Ion Channels in Mesangial Cells: Function, Malfunction, or Fiction

Ion channels in glomerular mesangial cells from humans, rats, and mice have been studied by electrophysiological, molecular, and gene-knockout methods. Two channels, a large, Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel (BK) and a store-operated Ca\textsuperscript{2+} channel (SOCC), can be defined with respect to molecular structure and function. Human BK, comprised of a pore-forming \(\alpha\)-subunit and an accessory \(\beta\)-subunit, operate as Ca\textsuperscript{2+}-sensing feedback modulators of contractile tone. SOCC have also been characterized in a mouse cell line; they are comprised of molecules belonging to the transient receptor potential subfamily.

A review of ion channels in mesangial cells (MC) must consider their multiple functions as contractile pericytes surrounding the glomerular capillary network. Determining these functions has been an especially difficult task in the case of MC, which are known for their ability to rapidly adapt to a variety of influences in the surrounding environment. The plastic nature of MC, in combination with their inaccessible glomerular location, has made it difficult to define their physiological role.

In the glomerulus, MC have many beneficial roles, which include production of growth factors that allow normal cell turnover, production of mesangial matrix to provide structural support for the capillaries, and modulation of glomerular hemodynamics through their contractile properties. However, when confronted with glomerular hypertension, inflammation, or other types of localized injury, MC may acquire a different phenotype. In some types of glomerular injury, MC often acquire characteristics of a myofibroblast, characterized by the production of \(\alpha\)-smooth muscle actin and interstitial collagens in addition to their normal matrix constituents (23).

Because of the difficulty in studying MC in vivo, their ion channel properties have been mostly studied after several generations of growth in a culture environment. The development of methods to isolate and grow MC in culture has enhanced our understanding of mesangial physiology to a level that occurs in immortalized cells, by the species or origin of the cells. However, when confronted with glomerular hypertension, inflammation, or other types of localized injury, MC may acquire a different phenotype. In some types of glomerular injury, MC often acquire characteristics of a myofibroblast, characterized by the production of \(\alpha\)-smooth muscle actin and interstitial collagens in addition to their normal matrix constituents (23).

Because of the difficulty in studying MC in vivo, their ion channel properties have been mostly studied after several generations of growth in a culture environment. The development of methods to isolate and grow MC in culture has enhanced our understanding of mesangial physiology to a level not possible with previous in vivo methods. However, the ion channels expressed in cell culture are very dependent on environmental influences and the species of origin. Thus large Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels are abundantly expressed in human MC in primary culture, but these channels have never been reported in rat MC in primary culture or in the immortalized mouse MC line.

The physiological relevance of ion channels may be suspect with all cultured cells. However, for MC in particular, it can be questioned whether the culture conditions are mimicking physiology, pathology, or an environment that is never found in vivo. A commonly used culture medium for growing MC includes 30 mM glucose. Such a high-glucose environment mimics the uncontrolled type 1 diabetic condition rather than normal physiology. Thus, as we create the best conditions for MC to thrive in vitro, we may be subjecting the cells to conditions that mimic disease or an entirely artificial environment. Furthermore, there is the question of whether any MC “line” truly mimics in vivo physiology. For example, although most MC lines proliferate readily, MC proliferation in vivo usually only occurs in pathological states. A study by Henderson and coworkers demonstrated the differences in ion channel functional expression in these two states by using MC from the \(H-2K^b-tsA58\) transgenic mouse in a series of patch-clamp studies (5–7). The \(H-2K^b-tsA58\) mouse is a simian virus 40 (SV40) mutant temperature-sensitive strain (tsA58), in which the oncogene is conditionally active (22). When the oncogene is active, MC proliferation mimics a cell line. However, when the oncogene is inactive, the growth rates are more similar to those found in vivo. Barber et al. (6) reported ATP-sensitive and small Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels only when the oncogene was turned on. Thus expression of these ion channels in MC may be affected by changes in phenotype that occur in immortalized cells, by the cell lines used, and by the species or origin of the cells.

Although it may have been initially beneficial to deemphasize in vivo methods, such as renal clearance and micropuncture, to develop a clear understanding of MC as they function in isolation, it is now imperative to return to in vivo experiments to understand whether our findings in cultured MC are relevant to a normal or diseased state. Although the inaccessibility of MC in vivo prevents the study of ion channels with the patch-
clamp technique, recently improved immunohistochemical methods and real-time PCR have been successfully used to identify and quantify the expression of plasmalemmal ion channels in the intact animal. In addition, the molecular ablation of genes, in combination with classical integrative approaches (i.e., clearance and micropuncture), has allowed the assessment of the physiological relevance of ion channels. Although the patch-clamp technique is a powerful tool, it is clear that the function of an ion channel in MC cannot be determined by this technique alone. This review will focus on all ion channels found to date in cultured MC as well as relate their physiological relevance as assessed by novel in vivo approaches. We wish to emphasize that patch-clamp technology has allowed only the initial identification of a variety of ion channels in cultured MC. The physiological function of these channels will not be fully understood until technologies have been developed to study the channels in their natural environment. Although a variety of channels have been described in various mesangial cultures by using patch-clamp techniques (summarized in Table 1), only two channels, the store-operated Ca\(^{2+}\) channel (SOCC) and the large-conductance, Ca\(^{2+}\)-activated K\(^{+}\) channel (BK), have been defined at the molecular level. We will therefore emphasize these channels and their physiological roles as we currently understand them.

### Anion Channels

Mallis et al. (33), using Cl\(^{-}\)-selective microelectrodes, demonstrated that MC chloride concentration was normally above electrochemical equilibrium but was reduced to near electrochemical equilibrium after the addition of bumetanide, a Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) transport inhibitor. This was an important discovery because it implied that Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) transport maintained the Cl\(^{-}\) concentration in MC above electrochemical equilibrium, that activating Cl\(^{-}\) channels would depolarize the membrane, and that Cl\(^{-}\) ions could play a role in regulating cell volume in MC. However, because these latter two actions would result in entirely different consequences to the cell, it might be more efficacious for the cell to divide these functions between at least two populations of Cl\(^{-}\) channels: one for membrane depolarization in response to a contractile agonist and the other for regulatory volume decrease when confronted with a hypotonic challenge. Evidence suggests that a nonrectifying Ca\(^{2+}\)-activated Cl\(^{-}\) current (\(I_{\text{Cl,Ca}}\)) is responsible for memb-

### Table 1. Channels discovered in mesangial cultures by using patch-clamp techniques

<table>
<thead>
<tr>
<th>Channels</th>
<th>G, pS</th>
<th>Activation</th>
<th>Selectivity</th>
<th>Function</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl(_{Ca})</td>
<td>4</td>
<td>Ca(^{2+})</td>
<td>I(^{-}) &gt; Br(^{-}) &gt; Cl(^{-}) &gt; F(^{-})</td>
<td>Depolarization</td>
<td>Rat, mouse (H-2K(_{B})-tsA58, oncogene inactive)</td>
<td>7, 12, 26, 46, 49</td>
</tr>
<tr>
<td>Cl(_{vol})</td>
<td>?</td>
<td>Low osmolarity</td>
<td>I(^{-}) &gt; Br(^{-}) &gt; Cl(^{-}) &gt; F(^{-})</td>
<td>Volume regulation</td>
<td>Human, SV40 murine cell line</td>
<td>12</td>
</tr>
<tr>
<td>NSC</td>
<td>25</td>
<td>Ca(^{2+})</td>
<td>Na(^{+}) = K(^{+})</td>
<td>Depolarization</td>
<td>Rat</td>
<td>35</td>
</tr>
<tr>
<td>SAC</td>
<td>40–76</td>
<td>Stretch</td>
<td>K(^{+}) &gt; Na(^{+})</td>
<td>Depolarization</td>
<td>Rat</td>
<td>13, 14</td>
</tr>
<tr>
<td>VOCC</td>
<td>11</td>
<td>Depolarization</td>
<td>?</td>
<td>Contraction, proliferation</td>
<td>Rat, human</td>
<td>18, 44, 47</td>
</tr>
<tr>
<td>ROCC</td>
<td>1</td>
<td>Ligand binding</td>
<td>Mn(^{2+}) &gt; Ca(^{2+}) &gt; Na(^{+})</td>
<td>Contraction, proliferation</td>
<td>Rat</td>
<td>27, 28, 34</td>
</tr>
<tr>
<td>SOCC</td>
<td>1–2</td>
<td>Store depletion</td>
<td>Ca(^{2+})-Ba(^{2+})</td>
<td>Store refilling</td>
<td>Human, rat, mouse</td>
<td>25, 29, 30, 32, 41, 45, 66</td>
</tr>
<tr>
<td>BK</td>
<td>206</td>
<td>Ca(^{2+}), depolarization</td>
<td>K(^{+})-Rb(^{+})-NH(_{4})(^{+}) &gt; Na(^{+})</td>
<td>Repolarization</td>
<td>Human</td>
<td>53, 59, 60</td>
</tr>
<tr>
<td>IK</td>
<td>40–65</td>
<td>Ca(^{2+}), depolarization</td>
<td>?</td>
<td>?</td>
<td>Rat, human</td>
<td>35</td>
</tr>
<tr>
<td>SK</td>
<td>?</td>
<td>Ca(^{2+})</td>
<td>?</td>
<td>?</td>
<td>Mouse (H-2K(_{B})-tsA58, oncogene active)</td>
<td>6</td>
</tr>
</tbody>
</table>

Cl\(_{Ca}\), Ca\(^{2+}\)-activated Cl\(^{-}\) channel; Cl\(_{vol}\), volume-sensitive Cl\(^{-}\) channel; NSC, nonselective cation channel; SAC, stretch-activated cation channel; VOCC, voltage-operated Ca\(^{2+}\) channel; ROCC, receptor-operated Ca\(^{2+}\) channel; SOCC, store-operated Ca\(^{2+}\) channel; BK, large-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel; IK, intermediate-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel; SK, small-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel; K\(_{ATP}\), ATP-sensitive K\(^{+}\) channel.
Each contractile agonist activated phospholipase C- via Gq coupling, thereby generating inositol trisphosphate and releasing Ca2+ from sarcoplasmic reticulum stores. However, Ca2+ released from stores is transient, lasting only a few minutes, whereas the enhanced Cl– conductance is a more sustained response. This question was subsequently addressed by Stepanovic et al. (57), who showed that the sustained Cl– conductance was the result of activating plasmalemmal voltage-gated Ca2+ channels, resulting in a long-lasting Ca2+ entry. Therefore, the initial release of Ca2+ from internal stores triggers an activation of I\textsubscript{Cl.Ca}, resulting in depolarization that activates voltage-dependent Ca2+ channels in the plasma membrane. The sustained Ca2+ entry maintains the depolarizing Cl– current to complete a positive-feedback loop (see FIGURE 1).

These early studies supporting a I\textsubscript{Cl.Ca} in MC were confirmed by Barber et al. (7) in patch-clamp experiments using the whole cell configuration in MC of H-2Kb-tsA58 transgenic mice and subsequently by single-channel analysis in the rat MC by Ling and co-workers (26). These investigators found that a 4-pS Cl– channel was activated in the cell-attached patch configuration either in response to ANG II or to thapsigargin, an agent that releases Ca2+ from stores by directly inhibiting the Ca2+-ATPase on the sarcoplasmic reticulum. It was concluded that these studies confirmed the presence and role of anion channels in glomerular MC, the data are consistent in their support of a Ca2+-activated Cl– channel that is involved in a depolarizing response initiated by contractile agonists. That such a Cl– conductance was involved in mesangial contraction was first shown by Okuda et al. (46), who found that angiotensin II (ANG II) and arginine vasopressin (AVP) induced a cell membrane depolarization associated with an input resistance that was sensitive to extracellular Cl–. The response to contractile agonists was mimicked by the Ca2+ ionophore A-23187, indicating that Ca2+ mediated the activation of the Cl– conductance. Hu et al. (20) subsequently showed with microelectrodes in rat MC that a Cl– conductance was evoked by endothelin-1, another potent stimulator of mesangial contraction (15, 56). Pavement (48) also showed that ATP evoked contraction of rat MC via activation of a Ca2+-dependent inward current that was mimicked by A-23187.

Although the Ca2+ dependency of I\textsubscript{Cl.Ca} was not in dispute, the source of Ca2+ entry was never clear. In all of these experiments, each contractile agonist activated phospholipase C- γ via Gq coupling, thereby generating inositol trisphosphate and releasing Ca2+ from sarcoplasmic reticulum stores. However, Ca2+ released from stores is transient, lasting only a few minutes, whereas the enhanced Cl– conductance is a more sustained response. This question was subsequently addressed by Stepanovic et al. (57), who showed that the sustained Cl– conductance was the result of activating plasmalemmal voltage-gated Ca2+ channels, resulting in a long-lasting Ca2+ entry. Therefore, the initial release of Ca2+ from internal stores triggers an activation of I\textsubscript{Cl.Ca} resulting in depolarization that activates voltage-dependent Ca2+ channels in the plasma membrane. The sustained Ca2+ entry maintains the depolarizing Cl– current to complete a positive-feedback loop (see FIGURE 1).

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**FIGURE 1.** Hypothesized relation among Ca2+-activated Cl– channels, VOCC, and BK in mesangial cells

IP\textsubscript{3}, inositol trisphosphate; PIP\textsubscript{2}, phosphoinositol bisphosphate; DAG, diacylglycerol; VOCC, voltage-operated Ca2+ channel; BK\textsubscript{Ca}, large-conductance Ca2+-activated K+ channel, [Ca2+]i, intracellular Ca2+ concentration.
small Cl– channels (called ClCa), initially activated by Ca2+ released from stores, depolarized the membrane to ensure a sustained Ca2+ entry that would initiate contraction and maintain the depolarizing effect of the inward Cl– current (26).

Volume-regulated Cl– channels

A study using the whole cell patch-clamp configuration in both human MC and a cell line from SV40-transformed mice provided evidence for a ICl.vol that is distinct from ICl.Ca (12). These investigators found that buffers that prevented an increase in intracellular Ca2+ uncovered an outward-rectifying Cl– current that was dependent on osmolarity (12). This volume-sensitive Cl– current may have been the same Ca2+-independent Cl– current that was identified (under both permissive and nonpermissive growth conditions) in MC harvested from H-2Kb-transgenic mice in an earlier study (7).

Pharmacologically, the ICl.vol could be distinguished from ICl.Ca. Employing cultured rat MC, they showed that a reduction in extracellular osmolality resulted in membrane depolarization that depended on the extracellular Cl– concentration. It is possible that ICl.vol is Ca2+-independent in human and mouse MC but is either Ca2+-dependent or not expressed in rat MC in culture.

Nonselective Cation Channels

Experimental evidence suggests that agonist-induced depolarization of the MC membrane involves not only Cl– channels but also nonselective cation channels (NSC). Matsunaga et al. (35) found that a 21-pS NSC in rat MC was activated by ANG II-induced increases in intracellular Ca2+ concentration. Thus activating NSC would depolarize the membrane potential and thereby activate voltage-gated Ca2+ channels, resulting in a sustained elevation in Ca2+ that could stimulate contraction.

Stretch-activated cation channels were discovered in cultured rat MC with both the whole cell and single-channel cell-attached configurations by either applying pipette suction or exposing the cells to hypotonic media (13, 14). The slope conductance was 40 pS with Na+ as the current carrier and 76 pS with K+ (14). It was suggested that the mechanosensitive cation channels could play a role in the response to stretch in the glomerulus.

Because of varying filtration fractions, the mesangium is exposed to alternating periods of cyclic stretching and relaxation. Stretch activation of NSC could depolarize MC, causing a contraction in response to elevated capillary pressures. It will be interesting to determine if these NSC are formed by the canonical transient receptor potential (TRPC) family, found to form Ca2+-permeable NSC in a variety of other cell types (63).

Ca2+ Channels

Cytosolic Ca2+ represents a convergent point of many signal-transduction pathways that modulate a diverse array of cellular activities ranging from contraction to cell growth. Although MC are contractile, they are also extremely responsive to cytokines and growth stimuli. Mesangial proliferation is a hallmark of a variety of renal diseases, including glomerulonephritis and diabetic nephropathy. The influx of extracellular Ca2+ is an important prerequisite for contraction and initiation of cellular DNA synthesis as well as mesangial proliferation. On the basis of gating mechanisms and biophysical and pharmacological properties, the channels mediating Ca2+ entry across the plasma membrane have been classified into three types: voltage-operated Ca2+ channels (VOCC), receptor-operated Ca2+ channels (ROCC), and SOCC. MC contain all three types of Ca2+ channels, only SOCC have been investigated thus far at the molecular level.

VOCC

VOCC, activated by membrane depolarization, were initially studied in cultured rat MC (62, 69). In these initial investigations, it was demonstrated with fluorescence microscopy that cytosolic free Ca2+ was increased by membrane depolarization with supraphysiological K+ concentrations (10–100 mM). Moreover, the basal Ca2+ concentration was enhanced by the L-type VOCC agonist BAY K 8644 and the response to depolarization was inhibited by nifedipine, a selective VOCC blocker (69).

Direct electrophysiological evidence for the presence of VOCC in MC was first presented by Nishio et al. in 1993 (44). With Ba2+ as a charge carrier, this group demonstrated in cultured rat MC an inward, whole cell current that was activated by membrane potentials more positive than –10 mV. The inward current was augmented by BAY K 8644 and was attenuated by nifedipine, suggesting that the current was an L-type VOCC. The L-type Ca2+ channel in MC was further verified in human MCs with single-channel analysis by Hall et al. (18), who used Ba2+ as a charge carrier to identify an 11-pS channel activated by depolarization in cell-attached
leading to activation of SOCC are mediated through G protein-coupled receptors that subsequently activate phospholipase C, resulting in production of inositol trisphosphate and diacylglycerol. The first evidence for SOCC in MCs was provided by Mene and co-workers (40), who used fura-2 fluorescence techniques in cultured human MC. These investigators described a novel Ca2+ entry pathway that was activated by depleting Ca2+ stores by either bathing the cells in Ca2+-free solution or by application of thapsigargin. That Ca2+ entry via SOCC was not abolished by nifedipine or verapamil and was not sensitive to plasma membrane depolarization suggested a channel different from VOCC. The initial findings of SOCC in human MC patches with BAY K 8644 in the pipette solution.

It has been suggested that VOCC play a role in both mesangial contraction and proliferation. In response to vasoactive peptides, mesangial contraction depends on sustained Ca2+ entry subsequent to membrane depolarization (26, 62, 69). Studies using specific blockers have also supported a role for VOCC in mesangial growth. Using cultured rat or human MC, nifedipine and benidipine have been shown to suppress [3H]thymidine and proline incorporation induced by fetal calf serum (47, 61).

ROCC

The receptor-operated Ca2+ entry pathway appears to be important for growth factor-mediated responses. In cell-attached patches of cultured rat MC, Matsunaga et al. (34) identified a Ca2+-permeable cation channel activated by PDGF in the pipette solution. This type of ROCC is distinguished from VOCC by a very low single-channel conductance of ~1 pS, with Ca2+ as the current carrier. The mesangial ROCC was cation selective, but divalent ions were only slightly more permeable than monovalent ions. Channel activation required PDGF in the patch pipette but not in the extracellular solution, suggesting a close coupling between PDGF receptors and the channel protein. Additional studies demonstrated that the protein tyrosine kinase inhibitor genistein attenuated the PDGF-evoked activation, suggesting coupling between the ROCC and tyrosine kinase receptors (27, 28). It has also been speculated that the mesangial ROCC may mediate endothelin-stimulated Ca2+ entry via a ligand-linked, G protein-coupled receptor (45). Thus the ROCC provides an alternative mechanism by which vasoactive and mitogenic agents regulate Ca2+-dependent MC responses, such as contraction and growth (27, 45, 51).

SOCC

SOCC open in response to depletion of the internal Ca2+ stores following activation of G protein-coupled or tyrosine kinase-based receptors. In MC, many vasoactive peptides that induce Ca2+ signals

FIGURE 2. Whole cell currents in response to application of thapsigargin

A: families of current responses to membrane voltage steps from -80 to 80 mV in 20-mV increments at 5-s intervals. Background currents have been subtracted from the currents generated 2 min after application of 1 μM thapsigargin (TG). B: current-voltage relations before and after TG. Currents 50 μs after establishing the whole cell configuration at all tested potentials were measured for analysis. *Significant difference before and after TG vs. corresponding test potentials; n = 8. C: percent change in whole cell currents induced by TG and blocking effect of La3+ on the response at holding potential of -80 mV. Currents before TG were normalized to 100% (control). *P < 0.05 vs. control and TG+La3+ groups. Adapted from Ref. 25, with permission.
were subsequently confirmed in mouse (67) and rat (45) MC. The electrophysiological and pharmacological properties of human mesangial SOCC were subsequently described by Li et al. (25), who used whole cell patch clamping, and by Ma et al. (32), who used a combination of fura-2 ratiometry and single-channel analysis (see FIGURE 2). Using Ba\(^{2+}\) as the charge carrier, a small inward current with a single-channel conductance of 2.1 pS (1 pS with Ca\(^{2+}\) as the charge carrier) was evoked by thapsigargin-induced store depletion. The single-channel currents were not affected by 5 \(\mu\)M Cd\(^{2+}\) but were inhibited by La\(^{3+}\), with an \(IC_{50}\) of 235 nM. With the use of fura-2 ratiometry, the SOCC was quantified as a rapid increase in intracellular Ca\(^{2+}\) concentration upon increasing the external Ca\(^{2+}\) concentration from nominally free Ca\(^{2+}\) to 1 mM. That the fura-2-measured Ca\(^{2+}\) increase was blocked by La\(^{3+}\) with an \(IC_{50}\) of 250 nM was corroborating evidence that the single-channel currents were SOCC. This small, 1-pS Ca\(^{2+}\) channel may be the same channel described as a PDGF-activated ROCC by Matsunaga et al. (34) (see above). Thus the same channel may be activated by ligand binding or release of Ca\(^{2+}\) from internal stores. However, the notion that the 1-pS channel is both a receptor and SOCC needs additional substantiation.

A variety of studies have shown that SOCC are involved in regulation and maintenance of intracellular Ca\(^{2+}\) stores. Also, altered regulation of SOCC can affect the contractile properties of MC. Such channels may play an important role in mesangial proliferative pathology of several diseases. Evidence suggests that the early "hyperfiltration" stage of diabetic nephropathy is partly the result of hyporesponsive contractile properties of MC resulting from altered Ca\(^{2+}\) signaling arising from a depression of SOCC (37, 39, 45). Moreover, SOCC-mediated Ca\(^{2+}\) influx has been considered a major component of the proliferative response of MC to agents such as ANG II, endothelin 1, bradykinin, and epidermal growth factor (11, 25, 31, 38), which are involved in renal diseases such as diabetic nephropathy and glomerulonephritis (36, 55).

Although attention has been focused on SOCC in the past decade, it is still not clear how SOCC are activated by store depletion. In human MC, some studies suggest that PKC-\( \alpha \) acts as a mediator to activate SOCC upon store depletion (29, 30). Application of PMA, a PKC activator, significantly enhanced SOCC in human MC in primary subculture (30). Inhibition of PKC-\( \alpha \) (either pharmacologically or with PKC-\( \alpha \) antisense) reduced SOCC current, suggesting that PKC-\( \alpha \) contributed to the PKC-mediated effects (29). These findings were supported by subsequent studies by Albert and Large (2, 3), who also demonstrated that PKC is a critical activator of SOCC in vascular smooth muscle cells, known to share similar phenotypic properties with MC.

Regulation of SOCC by store depletion in MC may be more easily investigated when the molecular makeup of SOCC is established. In all types of cells, the molecular identity of SOCC is a heavily debated topic. Many investigators have good evidence that members of the TRPC protein family, of which there are seven members (TRPC1–7), form the channels that mediate store-operated Ca\(^{2+}\) entry (64, 65). However, it is uncertain which of these seven members function as SOCC in any given cell. Facemire et al. (15a) revealed protein expression of TRPC1, 3, 4, 5, and 6 in rat glomeruli where MC reside. Consistent with these findings, a recent study showed that the mouse MC line expressed TRPC1 and TRPC4 mRNA and protein (66). However, immunocytochemical analysis showed that, whereas TRPC1 was cytoplasmically expressed, TRPC4 was localized to the plasma membrane (66) (see FIGURE 3). With the use of fura-2 ratiometry, the SOCC response can be quantified as the increase in intracellular Ca\(^{2+}\) concentration upon readdition of 1 mM Ca\(^{2+}\) to the bathing solution after store depletion with an agent such as thapsigargin. This method revealed substantial knockdown of the SOCC response in cells treated with TRPC4 antisense (66). Thus immortalized mice MC have SOCC that are, at least partly, formed by TRPC4 molecules.
K+-Selective Channels

The majority of studies of MC K+ channels have focused on BK in human MC in culture. Although other K+-selective currents have been reported in the various MC cultured preparations, information is incomplete regarding the regulatory properties and molecular identification of these channels. For this reason, this section will emphasize the MC BK.

BK

BK were first described in brain and smooth muscle, where these channels are particularly abundant. More recently it was shown that BK are also contained in glomerular cells, such as podocytes (42) and MC (59), where they may have a role in regulating glomerular filtration rate (GFR).

Because of their smooth muscle-like phenotype, it was not surprising that MC contained an abundance of BK. The relative ease in studying these channels in cultured human MC has led to considerable information regarding the subunit composition and regulatory properties of BK.

Also known as maxi K+ channels, BK are ~200-pS channels that are activated by both depolarization and increases in intracellular Ca2+ concentration. The composition of BK includes a constitutively expressed pore-forming α-subunit that coassembles with an accessory β-subunit. Presumably, α- and β-subunits form channels with a 1:1 ratio, although this is controversial (68). Four BK α-subunits are required for the formation of a completely functional channel that exhibits both Ca2+ and voltage sensitivity. However, the inclusion of a β-subunit alters the properties of the BK α-subunit. There are four known β-subunits, and each has a tissue-specific distribution (1, 9). For example, the β1-subunit is primarily found in smooth muscle, where it upregulates the Ca2+ and voltage sensitivities of BK α-subunit. In contrast, the neuronal β4-subunit dampens the voltage and Ca2+ sensitivities of BK α-subunit. In addition, the β-subunits are known to confer different kinase sensitivities to the channel (17, 19). These mechanisms provide for great variability in BK characteristics between cell types, allowing a constitutively expressed channel to be tailored to the specific requirements of the cell.

MC have many properties in common with smooth muscle cells, and like smooth muscle, MC express the BK α- and BK β1-subunits (24, 59). The human mesangial BK β1-subunit was sequenced (Pluznick and Sansom; GenBank accession no. AV515264) and found to be identical to BK β1 of smooth muscle. Although the specific subunits of the mesangial BK have been identified, the splice variants of the mesangial BK α-subunit have yet to be determined.

Paths leading to the activation of PKA, PKC, and PKG all regulate BK in various cell types (8, 17, 58, 70). However, for mesangial BK, the PKG pathway has been examined most extensively. In human MC, it has been demonstrated with single-channel patch-clamp techniques that BK are activated by PKG and cGMP as well as by soluble and particulate guanylyl cyclase stimulators such as nitric oxide (NO) and atrial natriuretic peptide (ANP), respectively (19, 58, 60). It was also demonstrated that activation of BK by these vasodilating agents is followed by a period of declining open probability (“rundown”). The effects of phosphatase inhibitors applied to single channels in the inside-out configuration suggest that the rundown phase is the result of dephosphorylation by protein phosphatase 2A (PP2A) (54). It appears, then, that the mesangial BK (or a tightly associated protein) is a common substrate for both endogenous PKG and PP2A, which act in opposition to fine-tune BK activity. Whereas BK play only a minor role to oppose constriction in some vascular beds (16), in MC, BK are a major component of the counteractive response to contraction (60). At least in part, this response is due to the Ca2+ sensitivity of BK. When an agonist causes the release of Ca2+ from stores, BK open in response to the increased intracellular Ca2+ concentration, thereby acting as a regulatory “brake” on the positive-feedback loop involving depolarization by V_{o U C C} and activation of VOCC (see FIGURE 1). However, in the case of some agonists—for example, ANG II—the increase in Ca2+ alone may not account for the magnitude of increased BK current (53). It therefore appears that some other (as yet unknown) factor also acts to open mesangial BK in response to agonist stimulation. One possible candidate is the multifunctional Ca2+/calmodulin-dependent kinase II (CaMKII) pathway, which is known to mediate a large proportion of the ANG II-induced activation of BK in MC (52). The application of KN62, a CaMKII inhibitor, in cell-attached patch-clamp experiments reduced the ANG II-induced activation of BK but did not affect the changes in intracellular Ca2+ concentration induced by ANG II (as measured by the fura-2 method). This implies that CaMKII may be an agent that “amplifies” the ANG II-induced signal to cause a greater BK feedback response in glomerular MC.

Although mesangial BK have been well characterized in vitro, it has been much more difficult to study their function and physiological significance using an in vivo model. In the glomerulus, MC are important contributors to the regulation of GFR (21). Therefore, as a responder to intracellular Ca2+, depolarization, and the kinase pathways, mesangial BK have an important role in modulating mesangial tone and thereby GFR.
The role of BK in the regulation of GFR has been explored using a BK\(\beta_1\)-null mouse model (BK-\(\beta_1\)/null), developed by Brenner et al. (10). Normally, when an animal is volume expanded, MC relax to increase the surface area available for filtration, thereby elevating GFR. Although BK-\(\beta_1\)/null mice have a normal GFR under basal conditions, they fail to elevate their GFR to the same extent as wild-type mice upon volume expansion (50). This suggests that the BK\(\alpha\)-\(\beta_1\) channel is an important regulator of MC tone and therefore GFR under the demanding conditions of volume expansion.

One potential activator of mesangial BK during volume expansion is the cGMP-PKG pathway, previously shown to activate BK in human MC as well as a variety of other cells (4, 43). Under conditions of volume expansion, at least two different stimulators of guanylyl cyclase—ANP and NO—would be expected to increase. One or both of these agonists may initiate MC relaxation by increasing the open probability of BK. That activation of BK by cGMP requires the presence of either a \(\alpha\)-\(\beta_1\) or a \(\beta_2\)-subunit explains the finding that the diuretic response to volume expansion is attenuated in BK-\(\beta_1\)/null mice (see FIGURE 4).

Alternatively, intracellular Ca\(^{2+}\) signaling may be involved in the mechanism of BK activation with volume expansion. Conceivably, an increase in volume causes an increase in pressure (shear stress) on the MC membranes, opening Ca\(^{2+}\) channels and increasing intracellular Ca\(^{2+}\). An elevation in Ca\(^{2+}\) would be expected to activate Ca\(^{2+}\)-sensitive BK if the increase was large enough and/or was localized near BK channels. Thus, because the BK \(\beta_1\)-subunit enhances the Ca\(^{2+}\) sensitivity of BK, the reduced capacity of BK-\(\beta_1\)/null to elevate GFR in response to volume expansion could be due to a decreased sensitivity to the Ca\(^{2+}\) entering the cell via stretch-activated cation channels in the absence of BK-\(\beta_1\).

**K\(_{\text{ATP}}\), SK\(_{\text{Ca}}\), and IK\(_{\text{Ca}}\) channels**

Matsunaga et al. (35) identified an intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channel (IK\(_{\text{Ca}}\)) in cultured rat MC by using single-channel patch-clamp techniques. This 40-pS channel was activated by intracellular Ca\(^{2+}\) as well as by AVP and ANG II. A similar 65-pS IK\(_{\text{Ca}}\) was found in human MC using single-channel analysis (53). This same study reported an small-conductance Ca\(^{2+}\)-activated K\(^+\) channel (SK\(_{\text{Ca}}\); 9 pS). However, both SK\(_{\text{Ca}}\) and IK\(_{\text{Ca}}\) channels were observed much less frequently than BK.

Barber et al. (6) found evidence for both ATP-sensitive K\(^+\) channels (K\(_{\text{ATP}}\)) and SK\(_{\text{Ca}}\) channels by using the whole cell patch-clamp technique in MC from the H-2Kb-tsA58 transgenic mouse. However, these currents were only observed when the tsA58 oncogene was active. When the oncogene was inactive, the only observed channel was osmotically sensitive and blocked by Gd\(^{3+}\).

**Summary**

Although a variety of ion channels have been studied in MC, the expression of these channels may be dependent on the species of origin and the culture conditions. Many of these channels have been shown to have functional roles in mesangial contraction, relaxation, and growth. However, culture conditions can simulate an environment that is physiological, pathological, or artificial. The future challenge will be to verify the physiological and pathological relevance of many of these channels in vivo by using integrative approaches in concert with appropriate knock-down and knock-in strategies.
References


