Glomerular Mesangial Cells: Electrophysiology and Regulation of Contraction

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Stockand, James D., and Steven C. Sansom. Glomerular Mesangial Cells: Electrophysiology and Regulation of Contraction. Physiol. Rev. 78: 723–744, 1998.—Mesangial cells are smooth muscle-like pericytes that abut and surround the filtration capillaries within the glomerulus. Studies of the fine ultrastructure of the glomerulus show that the mesangial cell and the capillary basement membrane form a biomechanical unit capable of regulating filtration surface area as well as intraglomerular blood volume. Structural and functional studies suggest that mesangial cells regulate filtration rate in both a static and dynamic fashion. Mesangial excitability enables a homeostatic intraglomerular stretch reflex that integrates an increase in filtration pressure with a reduction in capillary surface area. In addition, mesangial tone is regulated by diverse vasoactive hormones. Agonists, such as angiotensin II, contract mesangial cells through a signal transduction pathway that releases intracellular stores of Ca2+, which subsequently activate nonselective cation channels and Cl− channels to depolarize the plasma membrane. The change in membrane potential activates voltage-gated Ca2+ channels, allowing Ca2+ cell entry and further activation of depolarizing conductances. Contraction and entry of cell Ca2+ are inhibited only when Ca2+-activated K+ channels (BKCa) are activated and the membrane is hyperpolarized toward the K+ equilibrium potential. The mesangial BKCa is a weak regulator of contraction in unstimulated cells; however, the gain of the feedback is increased by atrial natriuretic peptide, nitric oxide, and the second messenger cGMP, which activates protein kinase G and decreases both the voltage and Ca2+ activation thresholds of BKCa independent of sensitivity. This enables BKCa to more effectively counter membrane depolarization and voltage-gated Ca2+ influx. After hyperpolarizing the membrane, BKCa rapidly inactivates because of dephosphorylation by protein phosphatase 2A. Regulation of ion channels has been linked casually to hyperfiltration during early stages of diabetes mellitus. Determining the signaling pathways controlling the electrophysiology of glomerular mesangial cells is important for understanding how glomerular filtration rate is regulated in health and disease.
I. INTRODUCTION

Glomerular mesangial cells (GMC) have been studied extensively with respect to their roles in the pathological development of long-term renal diseases. The histological progression of the mesangium in disease is typified by the nephropathy associated with diabetes mellitus (DM), which, in the early stages, is characterized by hyperfiltration and in the chronic stages by mesangial expansion and secretion of extracellular matrix. These pathological transformations in GMC ultimately lead to lethally reduced rates of filtration.

Glomerular mesangial cells both secrete and have receptors for a wide variety of hormones and growth factors, allowing multiple physiological roles in normal functioning glomeruli. By synthesizing and secreting an extracellular matrix, GMC provide structure and stability to the filtration barrier and have several functions, such as cytokine secretion and nitric oxide (NO) formation, that are similar to macrophages and endothelial cells. However, the embryologic origins and morphology of GMC are very similar, if not identical, to vascular smooth muscle cells.

Because of their position surrounding glomerular capillaries, early hypotheses proposed that mesangial cells influenced the capillary surface area. These hypotheses were based on experiments, in vivo and in vitro, showing that whole glomeruli contracted in response to vasoconstricting agonists (7, 56, 91, 192), such as angiotensin II (ANG II). These early studies were ultimately supported by experiments using cultured mesangial cells that responded like smooth muscle to vasoactive hormones (9, 195, 242).

In addition to supporting the capillaries structurally, glomerular mesangial cells secrete inflammatory and immune mediators. These signaling mediators are intraglomerular factors that coordinate the activities of a variety of target cells such as mesangial cells, epithelial podocytes, and invading macrophages to cause ultimately expansion of the mesangium and a decline in filtration rate. Discussion of proliferation and hypertrophy of mesangial cells and expansion of the extracellular matrix of the mesangium is relevant to the physiology and pathophysiology of the kidney but is beyond the scope of this review (refer to Refs. 128, 201).

It has been shown that GMC modulate the ultrafiltration of glomerular filtration rate (GFR) partly by changing the capillary surface area and partly by reproportioning blood volume in glomerular capillaries through the actions of mesangial loops. It is shown in the following sections that both mechanisms enable GMC to statically regulate GFR and may enable dynamic regulation as well.

For several years, mesangial cells have been known to regulate GFR, but only recently have studies focused on the electrophysiological determinants and signaling pathways modulating contractile tone. Several disorders, such as hypertension and diabetes mellitus, are characterized by dysfunctional regulation of GFR. Because GMC have been intricately involved in the pathological consequences of these disorders, it is important to study their hormonal regulatory processes. Several laboratories have employed electrophysiological methods, such as whole cell and patch clamping, to study regulation of mesangial contraction in cells cultured in both normal and pathological conditions. This review discusses in detail the findings regarding the electrophysiology, mechanics of contraction, and regulatory processes governing GMC tone in health and disease.

II. REGULATION OF GLOMERULAR FILTRATION RATE BY MESANGIAL CELLS

A. Ultrastructure and Function of the Glomerulus

As shown by the ultrastructure illustrated in Figure 1, the glomerulus contains capillary networks that allow the filtration of large plasma volumes at the renal corpuscle. After entering the glomerulus through afferent arterioles, plasma filters through fenestrated endothelial cells of the glomerular basement membrane (basal lamina), then through slit pores between podocyte foot structures into Bowman’s space. The filtration barrier (capillary endothelium, basal lamina, and slit pores) collectively filters the plasma, allowing only fluid, electrolytes, metabolic waste, small organic solutes, and small proteins to enter as filtrate into the nephron. Filtered blood exits the glomerulus through efferent arterioles.

In addition to the structural components of the filtration barrier, the glomerulus contains elements that regulate GFR. Advances in the micropuncture technique and the discovery of the Munich-Wistar strain of rat, which possesses experimentally accessible glomeruli near the surface of the renal cortex, have permitted direct measurements and mathematical modeling (58, 63) of the Starling forces governing ultrafiltration. These milestone discoveries, first reported by Brenner et al. in 1971 (34) (and recently recounted by Brenner et al., Ref. 33), showed that the filtration coefficient (Kf) could be modulated by vasoactive agents (23, 162). Thus GFR can be modulated by adjusting the pressure differential between the afferent and efferent arterioles or the functional elements that regulate the capillary surface area (96, 117).

The anatomy and fine ultrastructure of the glomerulus has been studied in detail to better understand the role GMC play in regulating GFR. The majority of glomerular capillaries bulge into Bowman’s space. The filtration barrier to Bowman’s space is defined as the endothelial cells that abut the glomerular basement membrane and podocytes that form a common surface covering a number
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of capillaries within the mesangium. The covering of the capillaries by the basement membrane is incomplete, forming a partial cylinder that is completed by GMC processes. Coupling of GMC to the basement membrane and the endothelial cells directly abutting the mesangium permits regulation of capillary surface area. Because there is no basement membrane between the endothelial cells and the mesangial extracellular matrix, the hydrostatic pressure within the capillary is equal to that in the mesangium. Thus GMC play an important role in regulating glomerular volume and countering the glomerular microaneurysms that result from increased capillary pressure.

Analysis of the glomerular ultrastructure shows that, in addition to completing the mechanical enclosure of the capillary, GMC also form loops that completely surround the capillaries (96). These mesangial loops regulate blood flow to the capillaries that they surround. Contraction of mesangial loops re propor tion blood volume within a capillary network and therefore affect glomerular hydrostatic pressure, ultrafiltration surface area, and GFR. Reproportioning of blood within the glomerulus is consistent with early studies demonstrating that both the mammalian (19, 66) and amphibian kidneys (189) had similarly adjustable rates of glomerular filtration.

B. Physiology of the Mesangial-Capillary Unit

Mesangial cells contain many of the same protein components as the smooth muscle contractile apparatus, including actin, myosin, and tropomyosin. However, it is unclear whether GMC and vascular smooth muscle cells contain identical isoforms of these proteins. Nakai et al. (163) reported that GMC do not express the predominant isoform of the smooth muscle myosin heavy chain. Moreover, Ishino et al. (100) identified non-smooth muscle-like contractile proteins in GMC. In contrast, Scheinman and Fish (199) and Ausiello et al. (9) reported that GMC con-
tain myosin isoforms identical to those of smooth muscle. Although more investigation is needed to clarify the expressed isoforms, it is clear that these cells contain a functional contractile apparatus. Interestingly, phenotypic changes of the contractile filaments are promoted by cyclic stretching and relaxation (81, 190, 249) as well as different culturing conditions (85, 110).

Although the contractile proteins are similar in smooth and skeletal muscle cells, GMC appear to contract through a mechanism more similar to smooth muscle. Mesangial contraction is initiated by voltage-gated Ca\(^{2+}\) entry and calmodulin-dependent activation of myosin light chain kinase. (Skeletal muscle contracts via Ca\(^{2+}\)-induced Ca\(^{2+}\) release and Ca\(^{2+}\)-dependent dissociation of troponin from actin.) In addition, similar to smooth muscle and unlike skeletal muscle, the contractile filaments of GMC are not rigidly arrayed. Kriz et al. (117) have shown that the microfilaments within GMC traverse the processes from one side to the other and are not aligned longitudinally. Actin-containing microfilaments predominate in mesangial pericapillary extensions that invade and couple the basement membrane and capillary endothelium to the cell. The cell body, however, contains few microfilament bundles.

Small bundles of extracellular fibrils in the mesangium form an extensive interwoven three-dimensional mesh that provides multiple contact points between GMC, the mesangial extracellular matrix, and the basement membrane. This large number of contacts permits GMC to support the mesangium and glomerular capillaries in addition to regulating capillary surface area. Fibronectin, the most abundant myofibril of the mesangium, functionally connects the noncontracting structure of the basement membrane and extracellular matrix with the contractile apparatus of the GMC. This creates a mechanical unit coupling opposing sites of the basement membrane. In this biomechanical unit, the basement membrane serves as the effector site while GMC serves as the contractile motor. This geometric structure suggests that contraction of GMC results in approximation of opposing parts of the basement membrane, which decreases the surface area of the capillaries as well as the rate of filtration.

Mesangial cells and the vascular smooth muscle of renal arterioles are complementary regulatory components of GFR. Intraglomerular hydrostatic pressure is established by the portion of systemic arterial pressure perceived by the glomerulus, which is modulated by the ratio of efferent to afferent arteriolar diameter. Normal intraglomerular hydrostatic pressure is 30–50 mmHg; however, capillary pressure can exceed 70 mmHg during glomerular disease. Hydrostatic pressure exerts on the wall of the glomerular capillary a distending force that is sensed by the mesangial-capillary unit. Under these physiological conditions, GMC integrate stretch stimuli and transduce them into isometric contraction (116, 117). This negative-feedback loop maintains GFR and counters mesangial expansion by reducing capillary surface area. These investigations of the fine ultrastructure of the glomerulus suggested static regulation of GFR. However, functional studies suggest that hormones, by their action on mesangial cells, modulate GFR in an intermittent fashion.

### III. HORMONAL ACTIONS

Several investigators have shown that isolated glomeruli could dynamically contract and relax (7, 86, 221), but Deng and Baylis (59) and Zatz and co-workers (188, 252) provided the most compelling support for the notion that hormonal regulation of mesangial contraction is essential for physiological control of GFR. These investigators showed that acute (59, 252) and chronic (188) blockade of glomerular NO synthase (NOS) decreased \(K_f\) and GFR by allowing contractile agonists to predominate. Thus, in this section, it is shown that GFR is maintained constant by the balance between the actions of contracting and relaxing hormones on glomerular mesangial cells.

#### A. Contracting Hormones

Myers et al. (162) were the first to examine the forces that develop glomerular ultrafiltration. Contracting agonists affect the hydraulic conductivity of the capillaries as well as GMC tone (64, 131). Coffey and Andrew (52) suggested that ANG II decreases hydraulic conductivity by constricting the foot processes of glomerular podocytes and narrowing the slit pore diameter.

Subsequently, other investigators showed that GFR is maintained constant in the presence of contractile agonists by autoregulation (22, 27). Contractile agonists tend to preferentially contract efferent over afferent arterioles. However, the elevated hydrostatic pressure in the glomerulus is countered by a simultaneous decrease in ultrafiltration surface area due to GMC contraction in response to a stretch reflex and the direct actions of contracting agonists (Fig. 2).

Tissue-culture techniques have enabled the direct study of contracting hormones on isolated glomerular mesangial cells. In general, these results have mimicked the mesangial cell in vivo; hormonal contraction is dependent on extracellular Ca\(^{2+}\) and proper excitation-contraction coupling (9, 67, 137). Hormones such as ANG II (136, 167, 168, 178, 179, 200) and arginine vasopressin (AVP; Refs. 9, 29, 70, 168) elevated intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and initiated contraction of cultured mesangial cells (9, 109, 200, 231). After these studies, intraglomerular ANG II and AVP binding were localized to both glomeruli (26, 36, 218, 221) and mesangial cells (44, 67,
of BQ-123 but not in the presence of IRL-1038 (87), suggesting that the signaling cascade was initiated by ET_B receptor binding.

Classical growth factors, such as platelet-derived growth factor (PDGF) and platelet-activating factor (PAF), have contractile as well as mitogenic effects on GMC (252). Moreover, insulin may have at least a permissive role as a mesangial contractile agonist. Platelet-activating factor increases Ca^{2+} (30, 62, 104) and initiates contraction (62) of monolayers of rat GMC. Insulin is required for normal Ca^{2+}-elevating and contractile responses to ANG II, PAF, and endothelin (62, 109).

B. Relaxing Hormones

Atrial natriuretic peptide (ANP) (57, 65, 74, 77) and NO (11, 24, 41, 183) are vasoactive hormones with important renal actions. The finding that ANP and NO relax GMC in tissue culture supports the notion that these dilating agents are physiological regulators of mesangial tone (216, 217).

Atrial-tissue extract as well as purified ANP have been associated with an increased GFR and a marked natriuresis and diuresis (38, 53, 88). Elevated GFR was partly attributed to increased capillary surface area and preferential relaxation of afferent over efferent arterioles (1, 35, 69). It was later found that ET-1 reduced glomerular surface area (12, 209) and was GFR (53, 69).

The in vivo effects of ANP were corroborated by several in vitro studies. Bianchi et al. (26) showed that isolated glomeruli, precontracted by ANG II, were dilated in the presence of ANP. Singhal et al. (216) found that ANP caused relaxation of isolated rat mesangial cells by elevating [Ca^{2+}] (12, 210–212, 250) via the phospholipase C (PLC)-inositol 1,4,5-trisphosphate (IP_3) signal transduction pathway (68, 214, 215). Thomas et al. (235) showed that the receptor was coupled to PLC by a pertussis toxin-sensitive G protein (235).

Specific endothelin binding to rat glomeruli and mesangial cells (214) was initially reported in 1989 (214). On the basis of binding and pharmacological studies, it was postulated that rat mesangial cells possessed at least two types of endothelin receptors (213, 230, 250). However, because ET-1 is also a potent stimulator of mesangial growth (215, 243), it has been difficult to determine which receptor is primarily involved in stimulus-contraction coupling. The receptor subtypes for ET-1 have been distinguished using affinity binding and the ET_A and ET_B receptor antagonists BQ-123 and IRL-1038, respectively. Yokokawa et al. (250) showed that BQ-123 suppressed elevations in [Ca^{2+}], in response to ET-1 in a dose-dependent manner. However, Hu et al. (87) found that a depolarizing Cl^- current, necessary to activate voltage-gated Ca^{2+} channels (VOCC), was activated by ET-1 in the presence of BQ-123 but not in the presence of IRL-1038 (87), suggesting that the signaling cascade was initiated by ET_B receptor binding.

Classical growth factors, such as platelet-derived growth factor (PDGF) and platelet-activating factor (PAF), have contractile as well as mitogenic effects on GMC (252). Moreover, insulin may have at least a permissive role as a mesangial contractile agonist. Platelet-activating factor increases Ca^{2+} (30, 62, 104) and initiates contraction (62) of monolayers of rat GMC. Insulin is required for normal Ca^{2+}-elevating and contractile responses to ANG II, PAF, and endothelin (62, 109).
which signals a mechanism to counteract the contractile tone on a Ca\textsuperscript{2+}-mesangial cells (141, 182, 207), NO activates cytosolic on Ca\textsuperscript{2+}
regulates GFR in response to specific humoral factors. A study by Stevanovic et al. (223) also confirmed that
effect is potentiated by cAMP (161). However, Nusing et al. contracted mesangial cells by a Ca\textsuperscript{2+}
mediation of voltage-gated Ca\textsuperscript{2+} channels. Membrane-soluble dibutyryl cGMP. However, basal levels of
Ca\textsuperscript{2+} were not affected, suggesting that ANP diminished Ca\textsuperscript{2+} influx or release from intracellular stores without affecting efflux or sequestration. These findings are consistent with studies performed in the laboratory of Hassid (83), who showed that ANP inhibits agonist-induced Ca\textsuperscript{2+} signaling in GMC.

Urodilatin, a peptide isolated from human urine, shares sequence identity with ANP except that four amino acids are added to the NH\textsubscript{2} terminus (204). Unlike ANP, Urodilatin is not found in the plasma and is probably synthesized primarily in the kidney. Urodilatin is 10-fold less potent in generating cGMP in mesangial cells; however, it is a more potent natriuretic than ANP and may also be important physiologically (197).

Endogenous NO also is an important mediator of renal blood flow and GFR (11). Glomerular mesangial and capillary endothelial cells both contain NO synthase; however, different isoforms are expressed (42, 207). Endothelial cells express constitutive NOS (cNOS), which is activated by cytokines and humoral-dependent increases in Ca\textsuperscript{2+}/calmodulin signaling. Glomerular mesangial cells express inducible, macrophage-like NOS (iNOS), which is modulated by cAMP-regulated transcription (71, 118, 119, 161, 180, 240). In addition, evidence suggests that some cytokines, such as interleukin-1 and tumor necrosis factor (119, 141, 182), activate iNOS in mesangial cells, and this effect is potentiated by cAMP (161). However, Nusing et al. (165) reported that prostaglandin (PG) E\textsubscript{2}, a physiological stimulator of cAMP, activated cyclooxygenase-2 but not iNOS (165). These results suggested that PGE\textsubscript{2} (via stimulation of cAMP) is not a physiological regulator of iNOS.

Although NO produced by cNOS regulates steady-state GFR, in mesangial cells (and macrophages), iNOS regulates GFR in response to specific humoral factors (183). Generated from either endothelial (142, 206) or mesangial cells (141, 182, 207), NO activates cytosolic guanylyl cyclase and increases intracellular cGMP, which signals a mechanism to counteract the contractile response.

The actions of contracting and relaxing agents on GMC in vivo and in vitro had been well described since the Munich-Wistar micropuncture and isolated glomeruli studies. However, the mechanisms of action were not elucidated until cells were grown in culture, allowing access for electrophysiological measurements. Several laboratories became involved in applying patch-clamp techniques to characterize plasmalemmal ion channels and ultimately describe the signal transduction pathways leading to contraction or relaxation of mesangial cells.

### IV. ION-SELECTIVE CHANNELS OF THE MESANGIAL PLASMALEMMAL MEMBRANE

Several distinct ion-selective channels have been discovered to play roles in modulating tone of glomerular mesangial cells. cultured mesangial cells have been used for the preponderance of earlier electrophysiological studies of ion-selective channels. More recently, immortalized murine and subcultured human mesangial cells have been used for determining the involvement of K\textsuperscript{+}-selective channels in particular.

#### A. Chloride Channels

Okuda et al. (168) were the first to show that Cl\textsuperscript{−} channels were involved in the regulation of mesangial tone. Using microelectrodes to record intracellular potential, these investigators found that ANG II and AVP induced membrane depolarization, which was mimicked by the Ca\textsuperscript{2+} ionophore A-23187. The depolarizations by ANG II, AVP, and A-23187 were all associated with decreased input resistances that were sensitive to extracellular Cl\textsuperscript{−} and the current-voltage relations reversed near the Cl\textsuperscript{−} equilibrium potential. These studies were subsequently corroborated by Hu et al. (87) who demonstrated in rat GMC with microelectrodes and ion substitutions that a Cl\textsuperscript{−} channel was involved in the depolarizing response to ET-1. In 1993, Pavenstadt et al. (175) showed that ATP contracted mesangial cells by a Ca\textsuperscript{2+}-dependent depolarization that was augmented by reducing extracellular Cl\textsuperscript{−}. Similar to the findings by Okuda et al. (168), an inward current was increased by A-23187. It was concluded that ATP contracted mesangial cells by releasing sequestered Ca\textsuperscript{2+} stores, which then activated plasmalemmal Cl\textsuperscript{−} channels and subsequently increased Ca\textsuperscript{2+} entry via activation of voltage-gated Ca\textsuperscript{2+} channels.

A study by Stevanovic et al. (223) also confirmed that mesangial contraction by ET-1 and AVP was dependent on Ca\textsuperscript{2+} influx through voltage-gated Ca\textsuperscript{2+} channels. Membrane depolarization preceded Ca\textsuperscript{2+} influx and was dependent on a Ca\textsuperscript{2+}-sensitive Cl\textsuperscript{−} conductance. These studies were consistent with the pharmacological studies of Hassid and co-workers (251) and Kremer et al. (113). The Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} conductance was also observed in the mesangial cells from the H-2K\textsuperscript{b}-tsA58 transgenic mouse by Barber et al. (17) using the whole cell patch-clamp technique.

Using cell-attached and excised patches of rat GMC in culture, Ling and co-workers (128, 129, 140) were the first to directly study the single-channel properties of mesangial Cl\textsuperscript{−} channels. These investigators described a 4-
pS, Ca$^{2+}$-activated Cl$^{-}$ channel that was activated in cell-attached patches by the addition of ANG II or thapsigargin, an agent that releases sequestered Ca$^{2+}$ stores. The concentration range of ANG II that activated the Cl$^{-}$ channel was the same as that reported to contract mesangial cells (82, 225, 231). It was therefore concluded that ANG II, through release of sequestered Ca$^{2+}$, activated this Cl$^{-}$ channel which then depolarized the mesangial cell membrane and opened Ca$^{2+}$ channels.

B. Nonselective Cation Channels

Craelius and co-workers (54, 55) characterized the biophysical properties of a 40-pS nonselective cation channel (NSCC) in cultured rat mesangial cells. This channel was activated by depolarization and mechanical stretch, suggesting a function as a mechanosensitive transducer. Activation of these channels may initiate isometric contraction in response to increases in intraglomerular capillary perfusion and transmural pressures.

A 25-pS nonselective cation channel was described in rat mesangial cells by Matsunaga et al. (146). Although the single-channel conductance was lower than that described by Craelius and co-workers (54, 55), all other biophysical properties were similar. This NSCC was activated by ANG II and AVP in cell-attached patches and by increasing Ca$^{2+}$ on the cytosolic side of inside-out patches. These properties led to speculation that the NSCC plays a role in depolarizing the membrane during agonist-induced elevations in intracellular Ca$^{2+}$.

C. Calcium Channels

In 1993, Nishio et al. (164) provided direct electrophysiological evidence for a voltage-gated Ca$^{2+}$ entry channel in mesangial cells. Using whole cell patch-clamp techniques, this group reported that depolarization of rat mesangial cells produced an inward barium current. Consistent with the pharmacology of L-type Ca$^{2+}$ channels, this current was activated by BAY K 8644 and inhibited by nifedipine. These results supported earlier studies by Yu et al. (251), who showed with fura 2 fluorescence that BAY K 8644 increased and nifedipine decreased resting Ca$^{2+}$ levels in rat mesangial cells. However, the single-channel characterization of these Ca$^{2+}$ channels has been elusive.

Since the initial whole cell studies, a possible Ca$^{2+}$ entry channel was described by Chen et al. (47), who reported a 21-pS Ca$^{2+}$-permeable, mechanosensitive channel in mesangial cells. However, because the voltage gating and pharmacological properties were not characterized, it is unclear whether this channel is responsible for the voltage-gated Ca$^{2+}$ entry of contraction. Similar to the NSCC described by Craelius et al. (55), this distinct Ca$^{2+}$-permeable channel may be involved in regulation of mesangial tone and GFR by transducing mechanical stress into isometric contraction.

A different Ca$^{2+}$ channel was identified in rat mesangial cells by Matsunaga et al. (145) and Ma and co-workers (132, 133). The 0.7-pS channel characterized in this report was highly selective for Ca$^{2+}$ and activated by pipette application of PDGF (132, 133). This channel was only slightly voltage dependent, and the effects of PDGF were sensitive to genistein, a receptor-linked tyrosine kinase inhibitor. Based on the local effects of PDGF and genistein sensitivity, these investigators suggested that it was a receptor-operated Ca$^{2+}$ channel (ROCC).

D. Potassium Channels

Most of the initial studies reported K$^{+}$-selective channels in cultured rat GMC using pharmacological tools in combination with fluorometric or whole cell current-clamp experiments (18, 107, 174). However, recent studies examined the properties of K$^{+}$-channels in cultured GMC from humans or from the H-2K$^{d}$-tsA58 transgenic mouse (18).

Properties of K$^{+}$-selective channels were first described by Matsunaga et al. (146), who found an intermediate 40-pS channel (IKCa) in rat mesangial cells in 1991. This channel was observed infrequently in cell-attached patches but was activated by AVP and ANG II, presumably by increasing [Ca$^{2+}$]. The properties of this channel were consistent with the notion that a Ca$^{2+}$- and voltage-sensitive K$^{+}$ channel could serve as a hyperpolarizing feedback regulator of voltage-gated Ca$^{2+}$ entry in contracted mesangial cells.

Studies on human mesangial cells were the first to show that large, Ca$^{2+}$-activated K$^{+}$ channels (BKCa) were important regulators of mesangial tone (224). Brayden and Nelson (31) had demonstrated in earlier studies that BKCa in vascular smooth muscle responded in a feedback manner to increases in [Ca$^{2+}$]i and depolarizing potentials (31). First described in human GMC in 1994 (224), BKCa were nearly quiescent in cell-attached patches when the cells were in normal physiological solution (135 mM NaCl and 5 mM KCl). Moreover, unlike BKCa in vascular smooth muscle, the response to agonist-induced contractions was very mild. However, basal BKCa activity was higher and the feedback response to ANG II was greater when the cells were in a solution containing 140 mM KCl (224). As is shown below, BKCa are significantly responsive as feedback regulators in physiological saline solution only in the presence of cGMP-elevating (vasorelaxing) agents such as NO and ANP. The mesangial BKCa shares sequence homology and biophysical properties with the product of the slo gene, which was cloned in the fly (8), mouse (2), and human (238).
TABLE 1. Ion channels of glomerular mesangial cells

<table>
<thead>
<tr>
<th>Conductance, pS</th>
<th>Selectivity</th>
<th>Activators</th>
<th>Inhibitors</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BKCa</td>
<td>205</td>
<td>K &gt; Rb &gt; NH4 &gt;&gt; Na = Cs &gt;&gt; Cl</td>
<td>Calcium, PKG, AA, MB</td>
<td>ChTX, lbTX, PP2A</td>
</tr>
<tr>
<td>SKCa</td>
<td>9</td>
<td>K &gt; Na &gt;&gt; Cl</td>
<td>Calcium</td>
<td>Apamin</td>
</tr>
<tr>
<td>KATP</td>
<td>65</td>
<td>K &gt; Na &gt;&gt; Cl</td>
<td>Cromakalin</td>
<td>4-AP, Glyb, ATP</td>
</tr>
<tr>
<td>IKCa</td>
<td>40</td>
<td>K &gt; Na &gt;&gt; Cl</td>
<td>Calcium</td>
<td>Apamin</td>
</tr>
<tr>
<td>NSCC</td>
<td>25–40</td>
<td>Monovalent &gt; divalent</td>
<td>Mechanical stress, calcium, insulin</td>
<td>Hyperglycemia</td>
</tr>
<tr>
<td>VOCC</td>
<td>21</td>
<td>Monovalent = divalent</td>
<td>Mechanical stress</td>
<td>Apamin</td>
</tr>
<tr>
<td>ROCa</td>
<td>NA</td>
<td>Ca = Ba &gt;&gt; Cl</td>
<td>Depolarization, BAY K 8644</td>
<td>Nifedipine</td>
</tr>
<tr>
<td>Cl</td>
<td>1</td>
<td>Mn = Ca &gt;&gt; Na &gt;&gt; Cl</td>
<td>Ras, calcium</td>
<td>Hyperglycemia, niflumic acid, NPPB</td>
</tr>
</tbody>
</table>

Two separate studies provided evidence for ATP-sensitive (KATP) and small, Ca2+-activated K+ channels (SKCa). In whole cell patch studies with immortalized mouse cells, Barber et al. (18) provided pharmacological evidence for these channels using apamin (SKCa) and glybenclamide (KATP). In a separate study, contraction of human GMC was induced by apamin and glybenclamide, suggesting that these channels are active at resting membrane potentials (195). With the use of the patch-clamp technique, K+-selective channels of 9 and 65 pS were found in human GMC at resting membrane potentials (195). These conductances are close to the SKCa and KATP channels in other tissues and may be the channels described by Barber et al. (18) using immortalized murine mesangial cells. However, because pharmacological experiments were not performed on the channels in human cells, it is difficult to conclude that these are the same SKCa and KATP channels as those described by Barber et al. (18) (see Table 1).

V. REGULATION OF MESANGIAL CONTRACTION

A. Signal Transduction Pathways

Early studies had demonstrated spontaneous (25) and ANG II-induced (86) contraction of whole glomeruli. However, the discovery of the Munich Wistar strain of rat, with surface glomeruli accessible for the micropuncture technique, facilitated experiments on the effects of a variety of contractile agonists on glomeruli in vivo. Using these rats, Ichikawa et al. (92) determined the direct association between vasoactive hormones and mesangial contraction (92) when they discovered that capillary surface area was dependent on Ca2+ and proper excitation-contraction coupling. Subsequently, the progression of tissue-techniques rapidly advanced our knowledge of the mechanism of regulating mesangial contraction. The initial studies of mesangial cells in culture revealed changes consistent with contraction in response to ANG II, AVP, and adenosine (9, 67, 137, 155). Since those studies, contraction was demonstrated in cultured GMC using a variety of vasoactive agents, including endothelium cell degeneration (150), endothelin (208, 209, 214), and PDGF (149). As shown by the experiments in vivo, extracellular Ca2+ was not necessary for contraction of cultured GMC.

Angiotensin II and AVP bind AT1a and V1a receptor subtypes, respectively (45, 157), which are coupled to PLC-β through heterotrimeric G proteins (51, 95). Upon activation of PLC-β by a contractile agonist, phosphatidyl-inositol 4,5-bisphosphate is catalyzed to 1,2-diacylglycerol (DAG) and IP3. The α-subunit of Gs and the βγ-subunit of Gi both regulate PLC-β activity (130, 222). It is therefore not certain which G proteins couple to PLC-β the multiple contracting hormone-receptor complexes in GMC. Studies show, however, that the agonist stimulus to contraction is sensitive to pertussis toxin, suggesting that Gs is the coupling protein (181, 202, 235).

Calcium is released from stores in the endoplasmic reticulum before Ca2+ permeability increases in the plasma membrane. Calcium entering the cell from the extracellular fluid complexes with calmodulin to activate myosin light-chain kinase (85), which phosphorylates the light chain of myosin and promotes interaction with actin (233). Cross-bridge formations mediate actin-myosin interactions, and tension is created by cross-bridge cycling. Contraction ceases when myosin light chain is dephosphorylated by protein phosphatase.
A. cAMP Signaling

The involvement of cAMP in mesangial relaxation was first demonstrated by experimentally manipulating synthesis of PGE2. Investigators found that inhibiting PGE2, which is normally increased in response to a contractile agonist (28, 153), results in potentiation of ANG II-induced contraction (90, 198). Other studies showed that several other factors that elevate cAMP also counteracted the effects of ANG II in vitro and in vivo (29, 198).

The cellular mechanism for the effects of cAMP on mesangial cells remained ambiguous partly because investigators reported conflicting results with respect to [Ca2+]i. Kurtz et al. (120) and Bonventre et al. (29) failed to observe an effect of cAMP signaling on agonist-elevated Ca2+ levels in GMC. In a separate study, MacDonell and Diamond (134) showed in rat aorta that cAMP signaling was not chronologically correlated with regulation of contraction. In contrast, several other investigators found that cAMP signaling decreased basal and/or attenuated agonist-induced elevations in Ca2+ concentration (62, 67, 84, 151, 170). The discrepancies between these studies may have been the result of using different experimental methodologies to quantify Ca2+ or the use of populations of GMC that were heterogeneous with respect to the capacity to contract. A subset of GMC may be unresponsive to cyclic nucleotide signal transduction (226). Alternatively, cAMP may influence GMC tone by either modulating the Ca2+ sensitivity of the contractile apparatus or influencing a parallel pathway signaling relaxation. It has also been suggested that cAMP, known to regulate sensitivity and contractility of cardiac muscle, modulates the Ca2+ sensitivity of the contractile apparatus in smooth muscle and ways signaling mesangial relaxation. Although contracting agents depolarize membrane potential, relaxing agents, such as ANP and NO, initiate signals that result in hyperpolarization and closure of VOCCs. Thus the ion-selective channels that set the membrane potential are the final targets for the relaxing pathways.

Electrophysiological investigations in conjunction with contraction assays and Ca2+ measurements using fluorescent dyes have begun to clarify the actions of relaxing agents on mesangial cells. In response to paracrine factors and diverse systemic hormones, mesangial relaxation is mediated, in large part, by the second messengers cAMP and cGMP.

B. Role of Ion Channels

Contraction of mesangial cells is dependent on the permeability of the membrane to Ca2+, which is tightly controlled by a variety of converging signal transduction pathways. Because some Ca2+ channels are sensitive to voltage, modulation of membrane potential is an important mechanism for regulating mesangial contraction. Thus cell entry of Ca2+ is the sum of the amplifying signaling pathways modulating membrane potential.

It was shown that contracting agonists such as AVP and ANG II increase intracellular Ca2+ in mesangial cells from basal concentrations of 80–120 nM to a peak of 0.5–1 μM (29, 83, 84). A secondary, sustained phase is dependent on extracellular Ca2+ and is inhibited by Ca2+ channel blockers and extracellular or intracellular Ca2+ buffers (84, 104, 231, 232, 251). The best evidence for a voltage-gated Ca2+ entry channel was provided by Nishio et al. (164), who produced an inward Ba2+ current that was activated by BAY K 8644 and inhibited by nifedipine. However, the mechanism for the sustained depolarization of the membrane potential was not clear, since the opening of only Ca2+ channels would not significantly affect the membrane potential.

Elevations in both Na+ and Cl− permeability provide the depolarization of membrane potential that results in sustained Ca2+ entry. Ling and co-workers (129, 140) provided the first electrophysiological evidence that Cl− channels were activated by ANG II and elevations in intracellular Ca2+. Ishikawa et al. (97) showed that increases in Na+ permeability also contributed to depolarization. The increase in Na+ permeability was probably the result of activating nonselective cation channels, which were activated by stretch, depolarization, and ANG II-induced increases in intracellular Ca2+ (54, 55, 146) (Fig. 3).

The ionic mechanisms for the contractile effects of growth factors have been characterized by Ling and co-workers using patch-clamp techniques. It was shown that insulin enhanced the response of Ca2+ and NSCC to ANG II (129). It was further proposed that the initial source of elevated [Ca2+], was through plasmalemmal 0.7-pS receptor-operated Ca2+ channels (ROCC) (133, 145). These ROCCs were activated by exposure to PDGF in the pipette and were sensitive to genistein, suggesting a receptor-linked tyrosine kinase.

VI. REGULATION OF MESANGIAL RELAXATION

The voltage-gating sensitivity of the mesangial VOCC imparts a major control point for the convergence of pathways signaling mesangial relaxation. Although contracting agents depolarize membrane potential, relaxing agents, such as ANP and NO, initiate signals that result in hyperpolarization and closure of VOCCs. Thus the ion-selective channels that set the membrane potential are the final targets for the relaxing pathways.

Electrophysiological investigations in conjunction with contraction assays and Ca2+ measurements using fluorescent dyes have begun to clarify the actions of relaxing agents in mesangial cells. In response to paracrine factors and diverse systemic hormones, mesangial relaxation is mediated, in large part, by the second messengers cAMP and cGMP.
GMC by inactivating myosin light-chain protein phosphatase (233, 246).

Activation of NOS may be a relevant parallel pathway for cAMP-mediated relaxation (161, 165). When generated from NOS catabolism of L-arginine, NO is a paracrine factor important in the regulation of glomerular inflammation and relaxation. However, signaling of NO in response to cAMP is a long-term regulatory process requiring from 4 to 12 h (161). Thus it is likely that crossover activation of the cAMP with the NO-dependent relaxing pathway is functionally relevant only during chronic, pathological regulation of mesangial tone. Crossover is probably not involved in intermittent regulation of the capillary microcirculation.

Ganz et al. (71) and Tolins et al. (236) provided evidence that Ca\(^{2+}\)-elevating agonists, such as acetylcholine and bradykinin, function to maintain mesangial tone in a manner similar to the actions of NO. Although both elevate Ca\(^{2+}\), these agonists initiated contraction only in the presence of NOS inhibitors. These results suggested that relaxation of GMC was a result of producing NO via Ca\(^{2+}\)-activated increases in NOS. Thus both cAMP- and Ca\(^{2+}\)-elevating hormones may influence chronic and intermittent regulation of GMC tone by stimulating NO synthase.

Another possible mechanism for the influence of cAMP on mesangial tone is the nonspecific activation of a component of the cGMP-dependent signal transduction pathway. In vascular smooth muscle, Lincoln and Cornwell (126) showed that cAMP induced relaxation by "crossover" activation of cGMP-dependent protein kinase. However, in human mesangial cells, cGMP-dependent kinase (PKG)-activated BK\(_{ca}\) are not crossover activated by cAMP (226) (see sect. viC). Thus it is not yet understood whether crossover activation is a physiological mechanism for relaxation signaling.

**B. cGMP Signaling**

Several studies have now shown that both ANP and NO attenuate agonist-induced increases in intracellular Ca\(^{2+}\) and contraction via the second messenger cGMP (4, 7, 84, 154, 155, 225). Thus cGMP signaling could modulate the activity of a protein or a transduction constituent that regulates Ca\(^{2+}\) influx by acting at several sites including 1) phospholipid signaling, 2) myosin light-chain kinase, 3) Ca\(^{2+}\) extrusion and resequestration, or 4) voltage-sensitive Ca\(^{2+}\) entry.

Guanosine 3',5'-cyclic monophosphate suppresses the activation of phospholipase in mesangial cells (20, 21). Moreover, both ANP and cGMP inhibit thromboxane and phorbol 12-myristate 13-acetate-stimulated protein kinase C (PKC) activity at steps distal to thromboxane receptor binding but proximal to PKC activation (229). Similar results were found in muscle, where the relaxation response to cGMP was attributed to inhibition of phospholipid metabolism (239). However, these results contrasted with those of Lincoln and Cornwell (126), who showed that cGMP signaling failed to affect phospholipid metabolism. Similarly, Kremer et al. (112) failed to note a change in PLC activity when ANP was added to GMC. Even if phospholipid signaling was affected by cGMP, GMC relaxation would not result from merely inhibiting lipid metabolism. Moreover, regulation by cGMP signaling of PLC activity in mesangial cells does not rule out the necessity of a mechanism for countering membrane depolarization and Ca\(^{2+}\) cell entry.

It is also uncertain how cGMP signaling affects cytosolic Ca\(^{2+}\) concentration and phosphorylation of myosin light-chain kinase in mesangial cells. Somatostatin is a cGMP-elevating hormone and potent inhibitor of ANG II. Garcia-Escribano et al. (73) found that somatostatin decreased GFR and reversed phosphorylation of myosin light-chain kinase in response to ANG II (73). However, the mechanism for the decreased phosphorylation has not yet been detailed.

Guanosine 3',5'-cyclic monophosphate could affect Ca\(^{2+}\) extrusion and resequestration by either 1) activating Ca\(^{2+}\)-ATPases, 2) inhibiting Ca\(^{2+}\) release from intracellular stores, or 3) blocking IP\(_3\) receptors (127). Some have observed that cGMP-elevating factors relax muscle by activating Na\(^+\)/Ca\(^{2+}\) exchangers and Ca\(^{2+}\)-ATPases (61). Mené et al. (152) reported that GMC contain a similar Na\(^+\)/Ca\(^{2+}\) exchanger, which is activated by [Ca\(^{2+}\)]. However, this mechanism functions only to decrease cytosolic Ca\(^{2+}\) by extrusion and resequestration and does not abolish entry via voltage-gated Ca\(^{2+}\) channels. Because Ca\(^{2+}\) entry is passive, with 1 \times 10^8 ions entering the cell per second, this mechanism could not efficiently be countered by antiporters and pumps dependent on hydrolysis of ATP. Thus solely activating extrusion/sequestration pathways would lead to a futile cycle of Ca\(^{2+}\) signaling and ATP consumption without a clear end point necessary for contracting cells to become quiescent. Moreover, sole activation of exchangers and ATPases in GMC is not consistent with the documented changes in membrane potentials in response to vasoactive compounds (43, 176, 225, 226). Thus it is possible that activation of exchangers and pumps by cGMP-dependent and -independent signal transduction pathways work in conjunction with mechanisms for attenuating Ca\(^{2+}\) cell entry. This mechanism would allow the extrusion and sequestration of Ca\(^{2+}\) to predominate and enable contracted cells to return to the resting state. Consistent with this notion is the feedback mechanism for controlling the transduction from an agonist-receptor complex to lipase generation. In this system, generated PKC inhibits phospholipase activity. After a contractile stimulus is initiated by receptor binding, the phospholipid signaling is controlled by feedback regulation.

Regulating the voltage-gated Ca\(^{2+}\) influx would be a
more precise mechanism for controlling contraction without disrupting other phospholipid-dependent pathways. In this way, Ca\(^{2+}\) signaling would be regulated at a site distal to and independent of phospholipid metabolism. Modulation of Ca\(^{2+}\) entry either directly, or indirectly through a feedback mechanism, is a possible point for regulating mesangial tone. Three possible mechanisms for regulating Ca\(^{2+}\) entry exist: 1) direct blockade of Ca\(^{2+}\) entry pathways, 2) inhibition of depolarizing conductive pathways, or 3) activation of hyperpolarizing conductive pathways. These latter two mechanisms could be accomplished by blockade of Cl\(^{-}\) and Na\(^{+}\) conductive pathways or activation of K\(^{-}\) channels. Either mechanism would hyperpolarize the membrane potential and inactivate voltage-gated Ca\(^{2+}\) channels.

Guanosine 3',5'-cyclic monophosphate directly inhibits Ca\(^{2+}\) channels in smooth muscle (98) and brain (123) but, to date, has not been observed to affect directly Ca\(^{2+}\) or any other cation channel in mesangial cells. However, cGMP signaling, via protein kinase, activates BK\(_{ca}\) channels in neurosecretory cells (244) as well as smooth muscle (160, 247) and mesangial cells (225).

C. Role of BK\(_{ca}\) Channels

Similar to other excitable cells, the resting membrane potential in GMC is set predominately by leak channels selective for K\(^{-}\) (114, 175, 195, 223). Increasing extracellular K\(^{+}\) depolarizes GMC and sustains voltage-dependent Ca\(^{2+}\) entry and contraction (115). Because activation of K\(^{-}\) channels hyperpolarizes both contracted and relaxed cells toward the K\(^{-}\) equilibrium potential (approximately −90 mV), K\(^{-}\) channels would function to both maintain the resting potential and counter contraction by regulating voltage-gated Ca\(^{2+}\) influx.

Ganz and co-workers (71, 102) showed that NO signaling relaxed GMC by stimulating a barium and charybdotoxin-sensitive K\(^{-}\) efflux. In response to NO signaling, intracellular K\(^{+}\) concentration decreased by >20 mM. In human mesangial cells, a BK\(_{ca}\) was characterized that was sensitive to both barium and charybdotoxin and shared biophysical and functional profiles with the BK\(_{ca}\) channel of muscle and neuron and molecular properties with the gene product of hslo (193, 224, 228) (Fig. 4).

The BK\(_{ca}\) have a variety of physiological roles in disparate cell types, including epithelia (76, 89), muscle (31, 194), brain (185), and GMC (224, 225); however, all have common biophysical properties, such as Ca\(^{2+}\) and voltage sensitivity. The mesangial BK\(_{ca}\) channel, which is inactive at rest, responds to membrane depolarization and Ca\(^{2+}\) signaling initiated by either contractile agonists or high ambient KCl. The biophysical characteristics of the mesangial BK\(_{ca}\) predict that (at resting [Ca\(^{2+}\)]) cells would have to be depolarized ~100 mV for significant channel activation. Similarly, at resting potentials, [Ca\(^{2+}\)] would have to reach millimolar levels before channel activation. This relatively mild voltage and Ca\(^{2+}\) sensitivity of the mesangial BK\(_{ca}\) contrasts with the same channel in smooth muscle, which is more active at rest and more responsive to signals of contraction (177, 194, 237). The relative quiescence of the mesangial BK\(_{ca}\) may be because of the action of an intracellular protein phosphatase (196). Thus the unstimulated mesangial BK\(_{ca}\) which is minimally activated in response to signals of contraction, is a low-gain feedback regulator of voltage-gated Ca\(^{2+}\) influx and thus contraction.

In response to NO and ANP, mesangial relaxation is partly dependent on stimulation of guanylyl cyclase and elevation of the second messenger cGMP. Although the mesangial BK\(_{ca}\) is not a good regulator of contraction at rest, the gain of the feedback response is significantly enhanced by cGMP signaling (224, 226). Either stimulation of cGMP synthesis by vasorelaxants or addition of exogenous dibutyryl cGMP activates BK\(_{ca}\) and hyperpolarizes GMC by 15–25 mV. Subsequently, BK\(_{ca}\) inactivate as membrane potential hyperpolarizes and Ca\(^{2+}\) entry is diminished (Fig. 5).

Thus transient activation of BK\(_{ca}\) hyperpolarizes the membrane potential and inactivates VOCC, Ca\(^{2+}\)-activated Cl\(^{-}\) channels, and NSCC, causing further hyperpolarization and cessation of contraction. However, BK\(_{ca}\) is only responsible for returning membrane potential to near the K\(^{-}\) equilibrium potential. Other K\(^{-}\)-selective channels are responsible for maintaining the resting membrane potential (195, 225). Because PKC feedback inhibits the contracting signals proximal to membrane depolarization, only a brief, transient hyperpolarization by BK\(_{ca}\) is necessary to abolish voltage-gated Ca\(^{2+}\) entry. Once Ca\(^{2+}\) entry is attenuated, extrusion mechanisms prevail.

The mesangial BK\(_{ca}\) is activated by a tightly associated, endogenous PKG (225, 226). Thus PKG, which also activates BK\(_{ca}\) of other tissues (5, 191, 234), plays an important role in relaxation of mesangial cells. However, unlike BK\(_{ca}\) of brain and uterine smooth muscle, mesangial BK\(_{ca}\) are not significantly regulated by protein kinase A (PKA) or PKC (226). Protein kinase A decreases and PKC increases the activity of BK\(_{ca}\) in rat brain cortex (50, 186, 187). In uterine smooth muscle, the cAMP-elevating vasodilator relaxin activates BK\(_{ca}\) via PKA (148). This suggests that tissue-specific activation by diverse kinases is a result of the development of specialized signaling pathways for regulating BK\(_{ca}\).

As with other phosphorylated enzymes and ion-selective channels, kinase phosphorylation of BK\(_{ca}\) is opposed by a dephosphorylating phosphatase (40, 78, 121, 122, 124, 125, 144, 147, 159, 166, 186, 187). As demonstrated in a recent study, the PKG phosphorylation of the mesangial BK\(_{ca}\) is specifically opposed by protein phosphatase 2A (PP2A) (196). Protein phosphatase 2A maintains mesang-
FIG. 4. Current tracings showing effects of NP, a nitric oxide (NO) donor, on large Ca\(^{2+}\)-activated K\(^+\) channels (BK\(_{Ca}\)) in cell-attached patches. Extracellular solution contained 135 mM NaCl and 5 mM KCl. Pipette solutions contained 150 mM KCl. Holding (cell) potential is 100 mV. Top trace: activation and subsequent inactivation of BK\(_{Ca}\) in response to NP. Note that amplitude of outward currents decrease as BK\(_{Ca}\) activate, membrane potential hyperpolarizes, and driving force (membrane potential minus K\(^+\) equilibrium potential) decreases. Bottom trace: NO does not affect BK\(_{Ca}\) in presence of guanylyl cyclase inhibitor LY-83583 (1 \(\mu\)M). These experiments support a role for BK\(_{Ca}\) to activate transiently and hyperpolarize membrane potential in response to exposure to NO. Inhibitory effect of LY-83583 suggests that NO does not directly affect BK\(_{Ca}\) but acts through stimulating guanylyl cyclase. [From Stockand and Sansom (225).]

Gial BK\(_{Ca}\) quiescent at rest and inactivates BK\(_{Ca}\) that are preactivated by PKG (196). These results explain the observation of transient responses of BK\(_{Ca}\) to cGMP and vasodilating agents but contrast to studies of BK\(_{Ca}\) in other cell types. White and co-workers (244, 245) reported that protein phosphatase is an intermediate component between PKG and activation of BK\(_{Ca}\) in neurosecretory cells. Zhou et al. (254) also concluded that BK\(_{Ca}\) of smooth muscle was activated by cGMP, which activated PP2A and dephosphorylated BK\(_{Ca}\). In contrast, PKG-dependent activation of mesangial BK\(_{Ca}\) is independent of an intermediate phosphatase (196) (Fig. 6).

Although mesangial BK\(_{Ca}\) are activated by PKG and inactivated by PP2A, definitive channel phosphorylation remains to be tested. Because PKG also activates Ca\(^{2+}\)-ATPases in smooth muscle (126, 184) and colocalizes with (135) and phosphorylates (37) cytoskeletal proteins, BK\(_{Ca}\) might be modulated by kinase/phosphatase regulation of an intermediary cofactor. However, PKG directly activates BK\(_{Ca}\) in inside-out patches (227), and recently, Alioua et al. (3) showed that PKG directly phosphorylates both the \(\alpha\)- and \(\beta\)-subunits of the heteromultimeric complex of BK\(_{Ca}\).

The cGMP-dependent protein kinase activates BK\(_{Ca}\) by resetting the thresholds for activation by voltage and Ca\(^{2+}\) (226). When the phosphorylation (PKG activation) limb of the kinase/phosphatase cycle predominates, the voltage activation threshold is lowered by as much as 76 mV, and the Ca\(^{2+}\) activation threshold is decreased by as much as 17-fold. Lowering the activation thresholds by these magnitudes at resting potentials initiates activation of normally quiescent BK\(_{Ca}\). Thus, in mesangial cells, PKG-dependent signaling increases the gain of feedback hyperpolarization by lowering the activation threshold of BK\(_{Ca}\) independent of voltage and Ca\(^{2+}\) sensitivities. A change in the threshold, but not sensitivity, would not affect the rate of feedback regulation but would increase the gain of the response. Thus, in the presence of vasorelaxing agents, intermittent regulation of \(K_t\) by ANG II would be less dynamic but would be sustained at a higher level.

In summary, activation of BK\(_{Ca}\) in response to contracting signals results in hyperpolarization of the plasma membrane, leading to subsequent inactivation of voltage-gated Ca\(^{2+}\) influx. This enables Ca\(^{2+}\)-ATPases and Na\(^+\)/Ca\(^{2+}\) exchangers to predominate, leading to a decrease in cytosolic Ca\(^{2+}\) with subsequent inactivation of Ca\(^{2+}\)-activated Cl\(^-\) and cation channels, and further membrane hyperpolarization. Contraction ceases. Atrial natriuretic peptide and NO increase the gain of this feedback system by decreasing the BK\(_{Ca}\) activation thresholds. The actions of dilating agonists are transduced by the second messenger cGMP, which specifically activates endogenous PKG. Protein phosphatase 2A suppresses mesangial BK\(_{Ca}\) activity at rest in addition to inactivating cGMP-activated BK\(_{Ca}\). This allows the GMC to return to rest and reset the feedback response.

VII. MESANGIAL CELLS IN DIABETIC MODELS

Several renal diseases are associated with mesangial dysfunction (for a recent review, see Bakris and Stein, Ref. 13). Many of these disorders are characterized by mesangial expansion and secretion of extracellular matrix, which contributes to dysfunctional glomeruli in the end stages of disease. However, in the early stages of DM, the rate of filtration can be increased as much as 100%
concentrates on studies that provide evidence for the involvement of electrophysiological pathways in diabetic models of mesangial dysfunction.

A. Mesangial Tone in Diabetic Models

Evidence for the involvement of mesangial cells in DM was first presented by Kimmelsteil and co-workers (93, 106), who showed that the mesangium was the initial glomerular site associated with diabetic nephropathy. Subsequent to these studies, it was shown that the hyperfiltration of early diabetes was associated with functional abnormalities of the glomerulus. First, Osterby et al. (173) showed that the fine ultrastructure of the glomerulus had an increased surface area in the early stages of diabetes. Second, Kikkawa et al. (105) found that isolated glomeruli from streptozotin-diabetic rats had diminished contractile responses. Third, contraction is considerably impaired when cultured mesangial cells are grown in an environment, such as insulin deficiency or hyperglycemia, that mimics DM (109, 111). Fourth, the agonist-elevated Ca\(^{2+}\) response in GMC is depressed in high-glucose environments (79, 99, 101). These results are consistent with the notion that the increase in capillary surface area is the result of dysfunctional regulation of GMC tone.

Some studies have not detected changes in glomerular capillary surface area in diabetic models (203, 253); however, these findings do not exclude a pathological role for mesangial cells in diabetic hyperfiltration. In the normal state, tonic contraction by mesangial cells counters intraglomerular hypertension to maintain GFR constant by autoregulation. In the diabetic state, hyperfiltration would result if mesangial cells could not isometrically and isotonically contract to counter intraglomerular pressure.

When glomerular cells are damaged, macrophages invade and initiate local inflammatory responses. Cytokines localized to the glomerulus activate iNOS and cause the production of NO (94). Because GMC relaxation is enhanced by NO, inflammatory cytokines will increase GFR because GMCs will no longer contract in response to transmural pressure. Hyperfiltration will enhance glomerular damage and cause fibrotic changes that lead to renal dysfunction. The notion that diabetic hyperfiltration is caused by cytokine-induced increases in NO was supported by Wu (248), who showed that the onset of diabetic hyperfiltration was delayed when NO production was inhibited.

The hyperglycemia of DM often produces hypervolemia associated with increases in natriuretic peptides (62, 171). Elevated ANP may contribute to hyperfiltration by activating BK\(_{\text{Ca}}\) channels and increasing the gain of the negative-feedback control system regulating mesangial tone.
B. Role of Ion Channels in Diabetic Models

High glucose levels encountered in diabetic conditions may affect the ability of mesangial cells to contract. As shown in rat mesangial cells, high glucose metabolizes to DAG, which activates PKC. Phospholipase A₂ is activated by PKC, leading to the release of arachidonic acid. Arachidonic acid and its metabolites activate BKCa channels of mesangial cells and other tissues. However, it is not certain that glucose increases arachidonic acid to levels high enough to affect BKCa channels.

Other studies implicated the dysfunctional regulation of NSCC and Cl⁻ channels in diabetic hyperfiltration. Seal et al. (205) showed with patch-clamp experiments that nonselective cation and Cl⁻ channels are suppressed in mesangial cells grown in high ambient glucose concentrations. They concluded that the decreased responsiveness of these channels was probably because of elevated PKC (activated by DAG), which inhibited PLC and resulted in an attenuation of the agonist-stimulated increases in IP₃ and Ca²⁺. Using the H-2KbtsA58 transgenic mouse, Barber and Henderson (16) found that the Ca²⁺-activated Cl⁻ conductance was diminished after passage 15. The conductance could be restored, however, by growing the cells with insulin. This study suggested that the normal contractile mechanism involving depolarization by Ca²⁺-activated Cl⁻ channels is impaired in DM partially because of the low insulin levels. Ling et al. (129) also found that insulin was necessary for the normal operation of Cl⁻ and NSCC channels in rat mesangial cells.

The consequences of suppressed regulation of NSCC and Cl⁻ channels would be prolonged hyperpolarization of membrane potential by BKCa, causing an attenuation of the effects of contracting agonists and an accentuation of the actions of relaxants. Thus evidence from a variety of diabetic models suggests that dysfunctional mesangial cells are partially responsible for the condition of diabetic hyperfiltration. However, it is likely that not one, but several signaling pathways and ion-conductive channels are involved in the pathological response of mesangial cells in DM.

VIII. CONCLUSION

Mesangial cells, integral components of the renal corpuscle, abut and surround the filtration capillaries within the glomerulus. With the consideration of recent studies in tissue culture together with earlier investigations of Starling forces, it is clear that there is a strong relation between the actions of hormones and the mechanisms regulating mesangial tone and GFR. Studies of the fine ultrastructure of the glomerulus indicate that the mesangial cell and the capillary basement membrane form a biomechanical unit capable of regulating filtration surface area as well as intraglomerular blood volume. Although structural studies suggest that mesangial cells regulate filtration rate in a static manner, functional studies of hormonal actions suggest that these cells play a more dynamic role, regulating GFR intermittently.

Like smooth muscle, GMC are contractile and possess the filaments actin and myosin. The contractile mechanism is also similar to that of smooth muscle, with Ca²⁺/calmodulin-activated myosin light-chain kinase instigating myosin-actin cross-bridge cycling. This mesangial excitability enables a homeostatic intraglomerular stretch reflex that integrates an increase in filtration pressure with a reduction in capillary surface area. In addition, mesangial tone is regulated by diverse vasoactive hormones. Ago-
nists contract mesangial cells through a signal transduction pathway that culminates in depolarization-induced Ca\(^{2+}\) cell entry.

The ion channels involved in the electrochemical coupling of agonist-induced contraction have been studied only recently. Similar to other excitable cells, the tone of mesangial cells is defined by the collective activities of plasmalemual conductive pathways, with a K\(^+\) conductance predominating at rest. Contractile agonists signal release of intracellular Ca\(^{2+}\) channels in unstimulated cells. When Ca\(^{2+}\) entry is perceived by VOCC and transduced into Ca\(^{2+}\) cell entry. Elevations in [Ca\(^{2+}\)], further activate depolarizing conductances, induce contraction, and enhance feedback desensitization of lipid signaling by PKC. Thus, after the initial stimulus, contraction is maintained by the electrical events at the plasma membrane, namely, voltage-gated Ca\(^{2+}\) entry (Fig. 7).

The entry of cell Ca\(^{2+}\) and the electrical events of contraction are inhibited only when mesangial cells are hyperpolarized toward the K\(^+\) equilibrium potential. This results when K\(^+\) channels are activated in the plasma membrane. One such channel, the mesangial BK\(_{Ca}\), is activated in response to both Ca\(^{2+}\) signaling and membrane depolarization. These properties allow BK\(_{Ca}\) to act as a negative-feedback regulator by hyperpolarizing the membrane in response to both the electrical and chemical signals of contraction.

The mesangial BK\(_{Ca}\) is a weak regulator of contraction in unstimulated cells; however, the gain of the feedback is measurably increased by the second messenger cGMP. Protein kinase G, activated by cGMP, decreases both the voltage and Ca\(^{2+}\) activation thresholds of BK\(_{Ca}\) independent of sensitivity. This enables BK\(_{Ca}\) to more effectively counter membrane depolarization and voltage-gated Ca\(^{2+}\) influx.

After hyperpolarizing the membrane and inhibiting Ca\(^{2+}\) influx, the mesangial BK\(_{Ca}\) rapidly inactivates due to dephosphorylation by PP2A. Thus PP2A, which maintains mesangial BK\(_{Ca}\) quiescent at rest, resets the negative-feedback mechanism regulating contractile tone. The physiological relevance of accelerated feedback is exemplified by experiments that show decreases in GFR when mesangial relaxation signaling is diminished.

Regulation of mesangial ion channels is not only relevant to the normal physiology of the glomerulus; dysfunctional regulation of mesangial tone also has been linked, albeit casually, to hyperfiltration during early stages of DM. It appears that aberrant regulation of several ion-selective channels may lead to dysfunctional control of mesangial tone and subsequent abnormalities in GFR. Hence, understanding the signaling pathways controlling the electrophysiology of glomerular mesangial cells is important for understanding regulation of renal filtration in human health and disease.

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