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Topical Review

Ion Channels in Renal Glomerular Mesangial Cells

R.M. Henderson, R.D. Barber
Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, United Kingdom

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Introduction

Renal glomerular mesangial cells have an important role in the maintenance of normal kidney function and in the processes that lead to glomerular pathology. They are multifunctional, partly resembling vascular smooth muscle cells, contracting in the presence of agents that influence glomerular filtration, and partly taking on the role of macrophages. They secrete and are the target for numerous inflammatory mediators and, since they also secrete matrix proteins, they are involved in the maintenance of the architecture of the glomerulus. The importance of mesangial cell function is thrown into focus in the features of a number of kidney diseases that are characterized by a proliferation of cells in the glomerulus which in turn is linked to an alteration in function of mesangial cells [26, 59]. In addition, there is evidence that mesangial cell function is altered in diabetes mellitus and this leads to hyperfiltration [43]. In recent years it has become evident that these functional and pathological changes may be intimately related to changes in ion channel function. Channels are involved not only in events leading to contraction of cells, but also have a role in the mechanisms leading to cell proliferation [15]. Depolarization of cells leads to elevated intracellular calcium levels which then triggers proliferation. This is highlighted by the case of angiotensin II, which causes depolarization and contraction in cultured mesangial cells, under apparently normal conditions, but may also cause mesangial cell proliferation [4]. The relationship between calcium signaling and cell proliferation is complex and has recently been reviewed [15]. The multifunctional role of the mesangial cell has made it difficult for the electrophysiologist to study, but work in recent years has meant that the role of ion channels even in the normal function of the mesangial cell is now becoming more clear. This review will center on the current state of knowledge of the types of ion channels expressed in mesangial cells and how their behavior may influence and be influenced by pathological conditions. Constraints of space necessarily limit the discussion and readers are encouraged to seek out other reviews that cover the various functions of the mesangial cell (including their electrophysiology) in more specific detail [39, 42, 43, 45, 49, 55].

Mesangial Cells are Complex and Multifunctional

Figure 1 shows a diagrammatic representation of the anatomical location of mesangial cells in relation to the other elements of the glomerulus. This anatomical relationship between mesangial cells and other glomerular structures is of crucial importance in fulfilling the multifunctional nature of the cells. Three major functions are attributed to mesangial cells:

(i) Mesangial cells make attachments with the glomerular basement membrane, and because of this they have been implicated in the control of glomerular filtration rate (GFR) [49]. Glomerular filtration is dependent on time and the ultrafiltration coefficient of the kidney

* *Present address:* 900, Preclinical Teaching Building, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA

Correspondence to: R.M. Henderson

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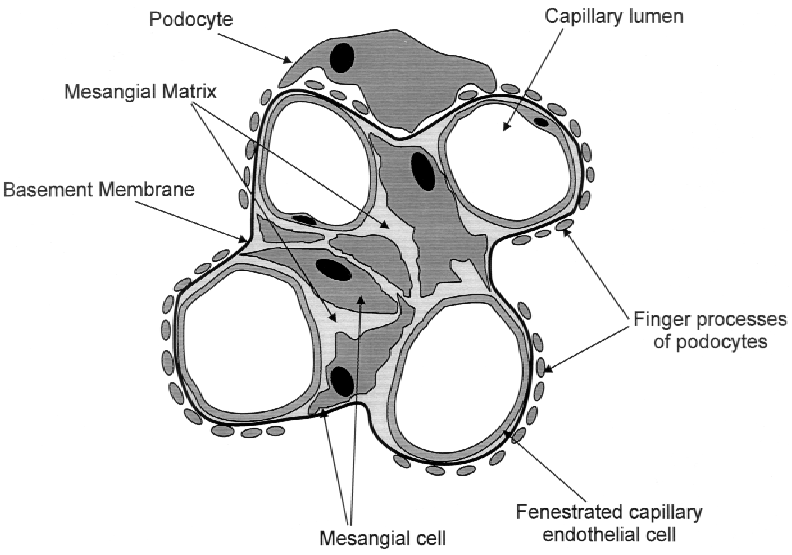

Fig. 1. A schematic diagram of the glomerulus showing the position of mesangial cells in relation to other structures. The three main cellular elements are the capillary endothelial cells (which form a fenestrated endothelial barrier); the irregularly shaped mesangial cells, and epithelial cells (podocytes). Primary processes extend from the podocytes and these in turn give off long, thin fingerlike projections which cover the capillary wall. The filtration barrier is composed of the fenestrated endothelium, the basement membrane, and the slits between the finger processes of the podocytes.

(k_f). This is proportional to capillary surface area and the hydraulic permeability of the capillary. It is thought that the active contraction of mesangial cells in the glomerulus causes a decrease of capillary surface area and that this will result in a reduction in GFR [49]. Initial observations in amphibian glomeruli [25] suggested that glomerular filtration is dependent upon the contractile elements in the glomerulus, the main candidates for this faculty being mesangial cells. Furthermore, in mammalian tissue, contraction of both glomeruli and mesangial cells has been observed in vitro (reviewed in [35]). In primary cultured or early passage mesangial cells contraction can be stimulated by numerous agents, among them angiotensin II (AII) and arginine vasopressin (AVP) [8]. Each of these hormones also reduces k_f in vivo [16, 31]. Similarly, parathyroid hormone and platelet-activating factor stimulate contractions in cultured mesangial cells, reduce mean plasma flow rate and increase total renal arteriolar resistance [30, 71, 73]. The contractions are rhythmic and synchronized and are reversible. They are largely dependent on the presence of extracellular calcium and can be correlated with specific binding of ^{125}I-labeled AII or [^{3}H] lysine vasopressin [8]. In explanted human glomeruli, the contractile cells have been located to the cells lining the capillary loops, the mesangial cells [14]. Compounds that cause contraction of mesangial cells in culture (and which indeed have a role in contraction of vascular smooth muscle) also reduce k_f in vivo. This evidence leads to the suggestion that mesangial cells are contractile in vivo and have a major role in the regulation of GFR[1]. Hormones, cytokines and growth factors cause a contraction in cultured mesangial cells. However, contraction is not the only response that these substances produce in cultured mesangial cells, other response include prostaglandin production and stimulation of mitogenesis. Furthermore, in the processes initiating these effects, most of these substances cause the mobilization of intracellular calcium stores, membrane depolarization and calcium entry. There is a wealth of literature describing the physiologi-

[1] There are three points that need to be borne in mind when considering this hypothesis. First, in a theoretical, biomechanical analysis of the glomerulus, Kriz and Kaissling [38] have suggested that the anatomical geometry and the structure organization of the glomerulus point to the mesangial cell fulfilling a static role. They suggest that mesangial cells maintain a residual tension to resist the distending forces acting across the capillary and perimesangial walls and thus prevent glomerular collapse. They also argue that even if the mesangial cell were to contract isotonically, it would not affect capillary diameter. Second, Kriz and coworkers [24] have shown that smooth muscle α-actin (SM-α actin) is found in cultured mesangial cells. SM-α actin is absent from mesangial cells in vivo and its presence, they argue, is the reason that mesangial cells are able to contract in culture. This is of some considerable importance since being relatively easy to isolate and to culture, most experimental work on mesangial cell function has been and still is conducted on cultured cells. Third, Steinhausen et al. [82] have shown that neither systemic nor local application of AII to superficial rat glomeruli in vivo gives rise to contraction.

cal actions and pharmacology of a wide range of these substances in mesangial cells, and a summary is given in the Table.

(ii) Mesangial cells have been shown to synthesize and secrete the components of the mesangial matrix as well as a wide array of compounds ranging from eicosanoids to peptide growth factors and cytokines, having both autocrine as well as paracrine functions [83]. The mesangial matrix has two major functions; to provide a robust, flexible support for the glomerular capillaries and to create channels for filtering and processing macromolecules. It is like other extracellular matrices, containing collagen (primarily Type IV), adhesive glycoproteins and proteoglycans which give it a tensile strength and high elasticity [49]. A side effect, incidental to the production of matrix proteins by the normal mesangial cell, is the difficulty in producing good gigaohm seals with a patch-clamp pipette onto the cells' surfaces, perhaps making progress in studies of ion channels in mesangial cells slower than it might otherwise have been.

(iii) It has been shown that approximately 3–7% of mesangial cells display macrophage-like properties. The mesangium is constantly perfused by the glomerular filtrate and this can contain potentially noxious macromolecules and filtration residues. The fate of these molecules is dependent upon their size and biochemical properties, but mesangial cells are responsible for the endocytic removal of the majority of these compounds and any other debris from the glomerulus [49]. The role of mesangial cells as macrophages is further supported by evidence that has shown that the cells express the inducible form of nitric oxide synthase [1, 79] and that they are able to produce oxygen radicals, cytokines, growth factors and eicosanoids [66].

Table. Summary of substances that produce elevationof free $[Ca^{2+}]_i$ and proliferation in cultured mesangial cells

Agent	Calcium mobilization	Mitogenic effects	References
Angiotensin II	√	√	[19, 44]
Arginine vasopressin	√	√	[5, 8, 17, 18]
Endothelin-1	√	√	[6, 32, 61, 77, 90]
Bradykinin	√	ND	[6, 12, 13, 88]
Histamine	√	ND	[76]
Adenosine	√	ND	[5, 33, 56, 57, 81]
5-hydroxytryptamine	√	√	[51, 52, 72]
β-Endorphin	ND	√	[78]
ATP	√	√	[2, 34, 63, 74]
Thrombin	√	√	[3, 7, 40, 87]
Thromboxane A_2	√	√	[50, 80]

(ND = not determined)

Agonist-induced Contraction of Mesangial Cells Involves a Change in Membrane Chloride Conductance and Calcium Entry

An early study to investigate the mechanism of mesangial cell depolarization was performed in cultured rat mesangial cells by Okuda et al. in 1986 [54]. Using conventional glass microelectrodes, they found that both AII and AVP caused a depolarization associated with a decrease in input resistance of the cell. The calcium ionophore, A23187, caused similar cell depolarization and the reversal potentials of these depolarizations, ionophore and hormone-induced, were dependent upon the extracellular chloride concentration. Since removal of bath calcium had no effect on the hormone-induced depolarization, the conclusion reached in [54] was that depolarization in rat mesangial cells is a result of the opening of calcium-activated chloride channels by calcium released from intracellular stores. These findings were supported and extended by Kremer et al. in 1989 [36]. Using a variety of ion substitutions, these authors confirmed that chloride movement was responsible for depolarization. However, even when the intracellular Ca^{2+} was buffered by 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), the authors were still able to obtain a depolarization in response to both AII and AVP. This indicated that depolarization could occur in both calcium-dependent and -independent manners. Similar calcium-dependent and -independent chloride conductances have been demonstrated using whole-cell recording in murine mesangial cells [9]. In their subsequent work, Kremer et al. showed that there are two separate mechanisms for the calcium-independent depolarization in rat mesangial cells, as well as the calcium-sensitive activation of the chloride current [37]. Of these two, one was shown to be G protein-mediated (experimentally activated by aluminum fluoride) while the other was due to the activation of protein kinase C. The conclusion is important since an elevation in intracellular Ca^{2+} activity ($[Ca^{2+}]_i$—whether as a result of release from intracellular stores or as a result of entry to the cell from the external medium via ion channels) can act as a messenger to stimulate intracellular pathways that produce cell proliferation as well as influencing the contractile mechanisms of the cell. Indeed calcium channel blockers have been shown to inhibit mesangial cell proliferation [23, 60].

Single chloride channel recordings in mesangial cells have been made by Ling and coworkers [41, 75] who have shown that calcium-activated chloride channels of 4 pS single-channel conductance are found in cultured rat mesangial cells. The open probability of these channels is increased by AII, presumably by its effects on $[Ca^{2+}]_i$ as thapsigargin (an inhibitor of the

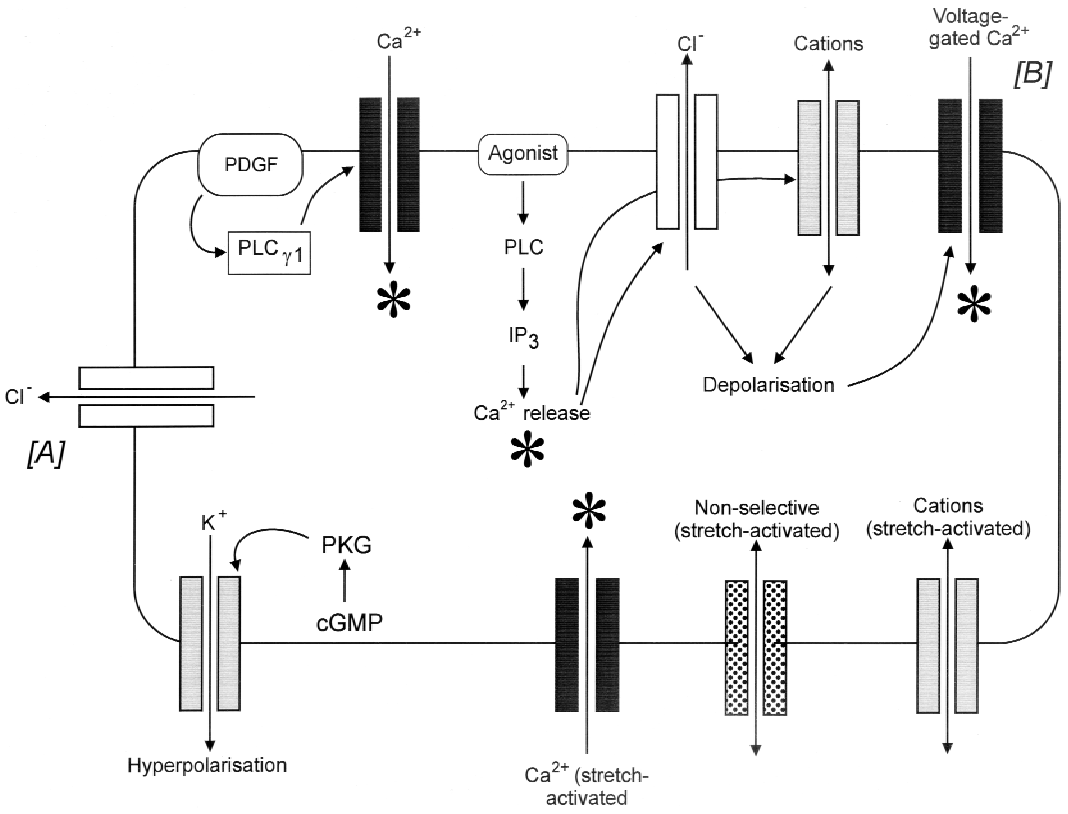

Fig. 2. A diagram showing the different types of ion channels that have been identified in cultured renal mesangial cells and which are described in the text. The calcium-independent chloride and voltage-gated calcium channels labeled *A* and *B* have been identified at the whole cell level, but the single channels underlying the currents have not so far been described. Most channels and conductances have been studied in cultured rat mesangial cells, but the potassium channel is the result of work on cultured human cells. Work on whole-cell conductances has indicated that ion channels in addition to those shown here are likely to be expressed in mesangial cells, and that this expression may be dependent upon the proliferative state of the cells [9, 10].

endoplasmic reticulum Ca^{2+}-ATPase) had the same effect. Ling and colleagues have also demonstrated that the channels are regulated by insulin, with the hormone increasing channel sensitivity to calcium [41]. Figure 2 shows a diagram of the mesangial cell representing these ion channels together with others described in this review.

Mechanical Stretch Activates Ion Channels in Mesangial Cells

Mesangial cells are closely apposed to the capillary loops in the glomerulus and as such are in an environment that is subject to changes in hydrostatic and hydrodynamic conditions. Stretch-activated and osmotically sensitive ion channels have been demonstrated to exist in a number of cell types, and given the contractile role of the mesangial cell it would not be surprising if channels existed in their membranes that allowed cell conductance to change as the cells are subjected to osmotic or mechanical stress. The first report of such channels, by Craelius et al., appeared in 1989 [21]. This group demonstrated nonselective channels with conductance of 62 pS that were activated by suction in cell-attached patches of cultured rat mesangial cells. In later work the same group demonstrated nonselective cation channels in the same cells that could be activated either by osmotic stress or by negative pressure [22]. These channels would presumably produce depolarization and mesangial cell contraction on stretching as a result of increased influx of Na^+. Other groups have also identified stretch-activated channels in mesangial cells. Pavenstädt et al. [65] demonstrated that a reduction in medium osmolality from 290 to 190 mosmol kg^{-1} produced cell swelling and a depolarization in primary cultures of rat mesangial cells and that the depolarization was mediated by a change in chloride conductance. Furthermore, simultaneous measurements in [65] using the Ca^{2+}-sensitive fluorescent ratiometric dye fura-2 during the osmotic challenge showed a rise in $[Ca^{2+}]_i$ coincident with the change

in chloride conductance. This rise was abolished in the absence of external Ca^{2+}, but was unaffected by the dihydropyridine voltage-gated Ca^{2+}-channel antagonists nicardipine, nifedipine or the phenylalkylamine verapamil. The single-channel basis of neither the chloride nor calcium conductances was studied in [65] but an earlier study [20] had identified Ca^{2+}-permeable, stretch-activated channels in rat mesangial cells. This channel had a conductance of 21 pS in 100 mM Ba^{2+} and its open probability was increased by either pipette suction or hyposmotic shock. The channel showed no sensitivity to nitrendipine and these findings indicate that it may underlie the calcium influx identified in [65].

If stretch is able to influence ion fluxes across mesangial cell membranes it is possible that chronic changes in the haemodynamic state of the glomerulus (which occur in several pathological states) might influence mesangial cell physiology. To test this hypothesis Harris et al. [28] have cultured rat mesangial cells on a flexible substrate under conditions of continuous stretch and relaxation, and compared their behavior with cells cultured on a rigid substrate. The cyclical stretch-relaxation changed the mesangial cells' morphology, enhanced their production of prostaglandins and matrix proteins and also produced a striking elevation in cell growth, which given the evidence from electrophysiological experiments, is likely to be related to an increased influx of calcium in the chronically stretched cells.

Calcium Can Enter Mesangial Cells by Both Voltage-dependent and -independent Pathways

Until recently, most of the evidence indicating that calcium channels are found in mesangial cells was indirect and came from studies using fluorescent indicators (such as fura-2) [57, 69], or those describing the effects of L-type calcium channel inhibitors on cell proliferation [23, 89]. The experiments described above [20, 65] suggest that the calcium channels involved in the stretch-activation of mesangial cells are not of the classical voltage-gated L-type channel. However, two reports that indicate that L-type calcium channels may be found in mesangial cells have been published. The first, [48], was still an indirect determination. It showed that uptake of $^{45}Ca^{2+}$ by mesangial cells can be stimulated by 50 mM potassium chloride depolarization or by the L-type dihydropyridine calcium channel agonist, BAY K 8644 (10 μM), and that uptake could be inhibited by the dihydropyridine Ca^{2+} antagonist nifedipine (10 μM) and verapamil (10 μM). Furthermore, isotope efflux from preloaded cells was stimulated by depolarization or by BAY K 8644 and inhibited by varapamil. The conclusion was that rat mesangial cells possess L-type calcium channels. Direct electrophysiological evidence comes from the work of Nishio et al. ([53], published in 1993, the same year as [48]) who, using the whole-cell configuration of the patch-clamp technique and 50 mM Ba^{2+} as a charge carrier, observed currents with characteristic current/voltage relationships consistent with L-type calcium channels. The current was inhibited by 1 μM nifedipine or 50 μM cadmium and augmented by BAY K 8644. Consequently, these authors too concluded that mesangial cells possessed L-type calcium channels. On the other hand, there are a number of other routes for calcium entry to mesangial cells. The BB-isoform of platelet-derived growth factor (PDGF), dissolved in a pipette solution containing 110 mM Mn^{2+}, was found to cause the activation of a small cation-selective channel in the cell-attached configuration of the patch-clamp technique [47]. With subsequent patch excision into a bath containing 110 mM Mn^{2+}, this channel was shown to have a very low unitary conductance of 0.67 pS and a selectivity, P_{Mn}/P_{Na} of 1.65. The PDGF receptor is a tyrosine kinase coupled receptor that acts by phosphorylation of phospholipase C-γ1 [15] with consequent production of inositol 1,4,5-trisphosphate (IP_3) and mobilization of intracellular calcium stores, thus providing a mechanism for stimulation of cell proliferation by growth factors. The PDGF-activated Ca^{2+} channel in mesangial cells has been shown to be under the influence of phospholipase C-γ1, and to be sensitive to both intracellular and extracellular calcium levels [67].

Besides the channels mentioned above and the mechanosensitive calcium-selective channel which has also been described ([20]—*see above*) a fourth possible route for calcium entry has been demonstrated by Matsunaga et al. [46]. These authors reported that cultured rat mesangial cells possess a calcium-activated, nonselective cation channel (NSCC) with single-channel conductance of 25 pS that displayed activation by AII and AVP. A similar (in all likelihood the same) channel, with a conductance of 27 pS, activated by AII has been described [41, 75]. In this latter study the channel was shown to be subject to regulation by insulin (*see below.*)

Potassium Channels May Hyperpolarize Mesangial Cells and Prevent Contraction and Proliferation

As with the chloride currents of mesangial cells, there have been several reports of measurements of whole-cell potassium currents. Pavenstädt et al. [62] have shown that extracellular ATP can stimulate both a chloride and a potassium current in rat mesangial cells and that 10 μM adenosine, acting on A_2 receptors, caused a membrane hyperpolarization by 14 mV via a mechanism involving cyclic AMP [64]. Another report of whole-cell potassium conductance is provided in [29] which shows that ET1 can also be responsible for the activation of potas-

sium as well as chloride currents. In conditionally immortalized mouse mesangial cells, a number of different potassium conductances have been isolated using whole-cell recordings on the basis of their pharmacology [10]. Single channel studies have however been few in number.

Noteworthy work on single potassium channels in mesangial cells has been published in a sequence of papers by Stockand and colleagues, the first appearing in 1994 [84]. In this paper the authors reported the presence in cultures of human mesangial cells large conductance (206 pS), Ca^{2+}-activated potassium channels. The channels showed similarity to the so-called 'Maxi-K' or 'BK_{Ca}' channels that have been reported in numerous cell types. Subsequent work has shown these channels to have an important role in mesangial cells.

If potassium channels were to be activated, then this would tend to hyperpolarize mesangial cells which would presumably counteract the tendency for the cell to contract. A number of agents including atrial natriuretic peptide (ANP) and nitric oxide (NO) inhibit the agonist-induced contraction of mesangial cells [58, 68]. Both these agents' actions are mediated by stimulation of guanylate cyclase with production of cyclic GMP (cGMP) and stimulation of the appropriate protein kinase. In the second paper of the sequence [86] Stockand and Sansom showed that the BK_{Ca} channels that they had identified in human mesangial cells displayed sensitivity in cell-attached patches to both nitroprusside (acting as a NO donor) and to ANP. The responses could be mimicked by the addition of the membrane-permeant dibutyryl cGMP, and at the cellular rather than the single-channel level, contraction of the cells induced by AII could be inhibited by both NO and ANP. The third paper of the sequence showed PKG to be the kinase responsible for activation of the BK_{Ca} channels [85]. Thus these channels appear to play a crucial role in the modulation of the contractile activity of mesangial cells by ANP and NO. Recent evidence [70] shows that the channels are down-regulated by protein phosphatase 2A after activation by the mechanism described above.

Diabetes Mellitus Predisposes Mesangial Cells to Alter Glomerular Hemodynamics by Changes in Ion Channel Function

As mentioned above Ling and colleagues have demonstrated that 4 pS chloride channels in rat mesangial cells are regulated by insulin [41]. The same is true of 27 pS nonspecific cation channels [41]. This is likely to have a bearing on the mechanism of glomerular hyperfiltration that occurs in Type I diabetes mellitus (where conditions of high plasma glucose coupled with low plasma insulin prevail). This point is addressed in summaries of their work by Ling and coworkers [42, 43]. The main points to note (which are described in detail in both these papers are that (i) AII receptor expression is diminished in the absence of insulin—reducing the capacity for AII-induced mesangial cell contraction; (ii) high extracellular glucose reduces the activity of both 4 and 27 pS channels. This may be due to inhibition of *myo*-inositol metabolism, with a consequent reduction in IP_3 production and resultant release of intracellular calcium stores—the effect can be overcome by addition of thapsigargin; (iii) insulin is needed for the effective activation of the 4 and 27 pS channels by AII. Murine mesangial cells, cultured in the absence of insulin also showed restoration of a calcium-activated chloride conductance when insulin was added to the medium [11]. The physiological consequence that would therefore occur with the conditions of low plasma insulin and high plasma glucose seen in type I diabetes mellitus would thus be a diminished ability of the mesangial cells to contract in response to agonist stimulation, with consequent increased K_f, GFR and resultant hyperfiltration.

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