Review

Regulation of mesangial cell function by vasodilatory signaling molecules

Lutz Buschhausen, Stefan Seibold, Oliver Gross, Tilman Matthaeus, Manfred Weber,* Eckhard Schulze-Lohoff

Department of Medicine and Nephrology, Merheim Medical Center, Medical Faculty of Cologne University, D-51109 Cologne, Germany

Received 6 December 2000; accepted 27 April 2001

Abstract

Proliferation of mesangial cells and expansion of mesangial matrix is a hallmark of glomerular disease leading to end-stage renal failure and requiring renal replacement therapy. Independently from the type of injury, e.g. in glomerulonephritis or diabetic nephropathy, the response to injury is remarkably uniform. Chronic glomerular disease is frequently associated with increases in systemic blood pressure and altered intraglomerular hemodynamics. Furthermore, reduction of systemic blood pressure and inhibition of the vasoconstrictor peptide angiotensin II have been shown to delay end-stage renal failure in various types of human kidney disease. Since vasoconstrictors of mesangial cells and efferent glomerular arterioles, such as angiotensin II, are thought to be detrimental for the progression of chronic glomerular disease, we propose that vasodilatory factors which antagonize the effects of angiotensin II, might have beneficial effects during the course of progressive kidney disease. To support this concept we will summarize currently available data on the role of vasodilatory signaling molecules such as natriuretic peptides (ANP, BNP and CNP), nitric oxide (NO), the prostaglandines PGE2 and prostacycline, and the purine mediator adenosine in the regulation of mesangial function. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Natriuretic peptide; Nitric oxide; Prostaglandine; Adenosine

1. Introduction

The majority of cases with end-stage renal failure is due to glomerular disease resulting either from diabetes mellitus or glomerulonephritis [1]. In these conditions, metabolic or immunologic injury causes damage of the glomerulus which is a specialized structure in the kidney serving to generate approximately 170 liters ultrafiltrate from the circulating plasma [1]. The glomerulus is built by four cell types: endothelial cells outlining the glomerular capillaries, mesangial cells functioning as pericytes adjacent to the glomerular capillary, visceral glomerular epithelial cells or podocytes attached to glomerular basement membrane, and parietal glomerular epithelial cells covering the inner surface of Bowman’s capsule [1]. Among these cell types glomerular mesangial cells are critical involved during various types of glomerular injury [2–4].

As part of the glomerular response to injury, mesangial cells undergo cell replication and increased production of extracellular matrix [2–4]. These processes have been shown to impair glomerular ultrafiltration finally resulting in glomerular sclerosis and end-stage renal failure. Because mesangial cells are critical involved in glomerular disease, much effort has been made to analyze mesangial cell function in normal conditions as well as in glomerular disease. To study the role of mesangial cell during progression of glomerular disease, cell culture of mesangial cells was extensively used over the last 20 years [2–4]. In addition, studies in experimental animals and human disease were used to examine the role of various factors which were thought to regulate mesangial cell function. Using these techniques, multiple cytokines, vasoactive peptides and autacoids were identified which stimulate mesangial cell proliferation as well as deposition of extracellular matrix within the glomerulus [1–4]. As detrimental factors these studies identified e.g. angiotensin

*Corresponding author. Tel.: +49-221-8907-3200; fax: +49-221-8907-3335.
E-mail address: e.schulze-lohoff@uni-koeln.de (E. Schulze-Lohoff).

Time for primary review 28 days.
Table 1

<table>
<thead>
<tr>
<th>Effect of the vasodilatory factors ANP, NO, PGE2 and adenosine on various signal transduction pathways and phenotypic changes in cultured mesangial cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Receptors detectable in cultured mesangial cells</strong></td>
</tr>
<tr>
<td><strong>Signal transduction pathways</strong></td>
</tr>
<tr>
<td>Ca²⁺↑, MKP-1↑, MAP-K↓, JNK↓, BKCa↑</td>
</tr>
<tr>
<td><strong>Phenotypic changes</strong></td>
</tr>
<tr>
<td>Relaxation ↑</td>
</tr>
<tr>
<td>Migration ↓</td>
</tr>
</tbody>
</table>

*Abbreviations: BKCa (Ca²⁺-activated K⁺-channels), IP3 (inositol trisphosphate) MAP-K (mitogen-activated-protein kinase), MKP-1 (mitogen-activated phosphatase-1), JNK (c-Jun NH2-terminal kinase), SAPK (stress-activated protein kinase).*

II. TGF-beta and PDGF to name only a few. Much less factors were identified which counteract the detrimental effects of these molecules [1–4]. Since natriuretic peptides, nitric oxide, vasodilatory prostaglandines and adenosine antagonize some effects of detrimental mediators such as angiotensin II, these vasodilatory signaling molecules may be potential therapeutic substances in progressive glomerular disease. Current information on the vasodilatory signaling molecules and their regulatory role on mesangial cell function is summarized in this review (Table 1).

2. Natriuretic peptides

The natriuretic peptides include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). These different peptides are encoded by separate genes which are regulated independently and exhibit specific tissue distribution. ANP is predominantly synthesized in atrial tissue of the heart. The full peptide of ANP is a 126 amino-acids polypeptide chain. In the kidney the propeptide is cleaved in a 32 amino-acids peptide called urodilatin (CDD/ANP-95-126) which is important for the regulation of volume and electrolytes [5]. Another cleavage product called cardiodilatin (CDD/ANP-99-126) generated in cardiac tissue is involved in blood pressure regulation [6]. The second natriuretic peptide, BNP, is a 32 amino-acids peptide which is generated from the propeptide by proteolytic cleavage. There are two active peptides derived from pro-CNP: the larger 53 amino-acids peptide and a smaller 22 amino-acids peptide.

Three different receptors recognizing natriuretic peptides have been identified. The type-A receptor has been found to bind ANP and -with lower affinity-BNP. The type-B receptor is specific for CNP [7]. The type-A receptor is found in larger blood vessels, adrenal glands and in the kidney. Cultured rat mesangial cells have been shown to express 12 000 ANP receptors per cell on the surface. Interestingly, the number of ANP receptors on the cell surface was increased by a low-sodium diet for 2 weeks, while a high sodium diet resulted in a decrease of receptor density [8]. The type-B receptor is expressed in the adrenal gland, the kidney and the brain. Cultured mesangial cells have been shown to predominantly express type-B natriuretic peptide receptors but also type A and C receptors [9]. Activation of type-A and type-B receptors by natriuretic peptides results in an activation of the cGMP signaling cascade which mediates the effects of natriuretic peptides. Type-C receptors for natriuretic peptides are thought to be involved in degradation of natriuretic peptides. After binding of natriuretic peptides to this receptor the peptide is internalized into the cell and degraded by proteolytic enzymes. The receptor is subsequently recycled to the cell surface for a new cycle of binding and re-endocytosis of natriuretic peptides [10]. In gastrointestinal smooth muscle cells type-C receptor is coupled to activation of phospholipase C-beta 3 via G-proteins and to inhibition of adenyl cyclase [11].

Since proliferation of mesangial cells is a hallmark of glomerular disease, the effect of ANP on mesangial cells has been examined in several studies (Table 1). While plasma concentrations of ANP in rats and humans are in the picomolar range [12], micro to nanomolar concentrations were required to produce activation of signal transduction pathways and phenotypic changes in cultured cells, i.e. in rat mesangial cells [13]. In cell culture ANP has been shown to inhibit the proliferation of rat mesangial cells as judged by 3H-thymidine uptake [14,15]. This effect was reproduced by addition of cyclic GMP analogue which indicates that ANP induced growth reduction is caused by an increase in intracellular cyclic GMP [16]. Inhibition of mesangial cell proliferation by ANP is partially due to increased expression of mitogen-activated-phosphatase (MKP-1). ANP and cyclic GMP analogues
have been shown to cause increased expression of MKP-1. MKP-1 is a negative regulator of mitogen-activated-protein-kinase (MAPK) which is a major mitogenic signaling molecule [17]. It has been shown that MAP-kinase activity induced by angiotensin II, endothelin I, PDGF and FGF is inhibited by ANP. As an example endothelin-1 activates the mitogen-activated protein kinases extracellular signal-regulated kinase (ERK) and c-Jun NH2-terminal kinase (JNK) associated with mesangial cell proliferation. ANP and BNP inhibited ET-1-induced activation of ERK and JNK in a dose-dependent manner [18–20]. Addition of HS-142-1 reverses the antimitogenic effect of ANP, indicating that the ANP effect is mediated by specific ANP receptors [21]. The inhibitory effect of ANP is even stronger and reaches 70% inhibition if the type-A receptor of natriuretic peptides is overexpressed. Direct transfection of ANP in these cells causes not more than 20% inhibition. The effect was reversed by addition of an inhibitor of cGMP dependent protein kinase [22]. Furthermore, ANP has been shown to inhibit mesangial cell contraction induced by angiotensin II [23]. Kohno et al. demonstrated that ANP and BNP inhibited the migration of mesangial cells through a cGMP-dependent mechanism [24].

In addition to activation of MKP-1, ANP may inhibit mitogenesis of mesangial cells by upregulation of the anti-mitogenic factor TGF-beta. Increased expression of TGF-beta is due to increase in cyclic GMP after stimulation with ANP. The anti-mitogenic effect of ANP is partially reversed by addition of neutralizing anti-TGF-beta antibodies [25]. Since ANP induces TGF-beta in cultured mesangial cells, ANP may increase matrix deposition in the mesangium.

In cultured rat mesangial cells ANP reduces the increase of intracellular Ca\(^{++}\) by a cyclic GMP-dependent mechanism [26]. Ca\(^{++}\) is an important cation involved in many cellular functions. Ca\(^{++}\) binds to a number of proteins and thereby regulates the structural integrity of cells, their contractility and the production of inflammatory mediators. The effect of ANP on intracellular Ca\(^{++}\) is dependent on cell density. An increase in cell density results in a pronounced decrease in intracellular Ca\(^{++}\) [26]. In addition it has been shown that release of intracellular Ca\(^{++}\) by angiotensin II is inhibited by ANP [27]. It is thought that the effect of ANP on intracellular Ca\(^{++}\) concentrations plays an important role in ANP mediated vasodilatation [28].

ANP has been shown to activate large Ca\(^{++}\)-activated K\(^{+}\)-channels (BKCa) via formation of cGMP and a cGMP-dependent protein kinase in rat mesangial cells. BKCa appears to play an important role in countering the angiotensin II induced contraction of mesangial cells. Angiotensin II causes a depolarization of the membrane by activation of cation channels and voltage-gated Ca\(^{++}\)-channels leading to additional Ca\(^{++}\) influx. BKCa prevents Ca\(^{++}\) influx by supporting repolarization of the plasma membrane [29–31].

Intracellular pH has been shown to be involved in the complex processes leading to cell replication. The natriuretic peptides inhibit the Na\(^{+}\)/H\(^{+}\) exchanger in mesangial cells which impairs pH-regulation in these cells. The effect is dependent on cyclic GMP and inhibited by a protein kinase G-inhibitor [32].

Similar to ANP the natriuretic peptide CNP has been shown to be antimitogenic for cultured mesangial cells. Injection of CNP into rats caused a significant reduction of mesangial cell proliferation in mesangioproliferative glomerulonephritis in rats. Furthermore, deposition of collagen type IV and fibronectin was reduced in this model [33]. Similarly, mice overexpressing brain natriuretic peptide (BNP) exhibit ameliorated glomerular injury in a model of focal glomerular sclerosis [34].

### 3. Nitric oxide

Since the discovery of nitric oxide (NO) as endothelial derived relaxing factor (EDRF) in the late 1980s, the role of NO in renal, glomerular and mesangial function was extensively studied [35]. NO is a small, short lived molecule which is produced from l-arginine by a family of enzymes called NO synthases [36]. The type-I NO synthase is found in neuronal cells of the brain and the macula densa of the kidney where NO is thought to play a role in tubuloglomerular feedback [37]. The inducible form of NO synthase (type-2 enzyme) is found in macrophages and glomerular mesangial cells. It is induced by bacterial endotoxin and cytokines (e.g. TNF-alpha, interleukin-1-beta, interferon-gamma). This isoform has been described to produce NO at much higher concentrations than the other isoforms. Isoform three of NO synthase has been constitutively expressed in endothelial cells throughout the body. NO has been shown to activate soluble guanylyl cyclase which results in a strong increase in intracellular cyclic GMP formation in glomerular disease. NO production has been shown to be greatly increased in the glomerulus [38]. Infiltrating macrophages stimulated by cytokines are thought to contribute to strong NO production found in this condition. Narita et al. proposed that high levels of NO may even mediate immunologic injury to kidney mesangium in experimental glomerulonephritis [39]. By contrast, endogenously synthesized NO has been shown to prevent endotoxin induced glomerular thrombosis [40]. Recently, expression of the three isoforms of NO synthase was studied in renal tissue of patients with different types of glomerulonephritis. Inducible NO synthase was localized in mesangial cells, glomerular epithelial cells and infiltrating macrophages in the diseased glomeruli, whereas immunostaining was hardly detected in normal human kidney. Endothelial NO synthase was present in glomerular endothelial cells and endothelium of cortical vessels in control and diseased kidney. The extent of staining for endothelial NO synthase correlated negatively with the
degree of glomerular injury while the extent of staining for inducible NO synthase correlated positively with the degree of glomerular injury in the same tissue. The brain form of NO synthase was not detected in normal and nephritic glomeruli [41].

To study the role of NO in glomerular pathology in more detail, several authors analyzed production of NO by cultured mesangial cells as well as paracrine and autocrine effects of NO on this cell type (Table 1). Garg and Hassid found that NO donors dose-dependently inhibited cell replication of cultured mesangial cells which was thought to be due to increased intracellular cyclic GMP [42]. Accordingly, NO donors were found to reduce angiotensin II-induced contraction of rat mesangial cells [43]. In afferent arteriolar smooth muscle cells angiotensin II is mitogenic and this effect is inhibited by NO [44,45]. Furthermore, exogenous nitric oxide inhibited mesangial cell adhesion to extracellular matrix molecules [46]. High levels of nitric oxide were found to cause apoptotic cell death of rat mesangial cells [47]. Recent, Huwiler and Pfeilschifter demonstrated that exogenous NO stimulated stress-activated protein kinase [48] in mesangial cells. As observed by immunohistochemical studies in human tissue and experimental animals, the inducible form of NO synthase is strongly upregulated in glomerular inflammation both in mesangial cells as well as in macrophages. Upregulation of inducible NO synthase is regulated by several factors including interleukin-1-beta, TNF-alpha and cyclic AMP in cultured mesangial cells [49]. On the other hand, stimulation of inducible NO synthase is inhibited by transforming growth factor-beta [50], vasopressin [51] and extracellular nucleotides [52].

Recently, NO has been shown to play a role in regulation of gene expression [53]. In human mesangial cells, NO-donors induced upregulation of COX-2 expression for up to 24 h, followed by inhibition of COX-2 expression subsequently. This effect was due to upregulation of NF-kappa B caused by NO [53].

Due to its pleotropic effects the precise role of NO in glomerular disease is not clear at the present time. Cytotoxic effects described in several studies and promotion of glomerular hyperfiltration indicate that upregulation of NO production may be detrimental in glomerular disease. On the other hand, inhibition of mesangial cell proliferation and reduction of platelet and leukocyte adhesion in glomerular capillaries may represent beneficial effects during the course of glomerular disease. Because NO is a very short lived signaling molecule, space and time relationship of NO production may determine the role of NO in specific types of glomerular disease.

4. Vasodilatory prostaglandines

Prostaglandin E2 (PGE2) has been shown to inhibit proliferation [54] and vasoconstriction [58] of cultured mesangial cells (Table 1). These effects are mediated by cyclic AMP and by activation of protein kinase A [54]. Cyclic AMP inhibits the activation of mitogen-activated protein kinase (MAPK) in mesangial cells [55]. This effect was found to be blocked by indomethacin. The inhibitory effect on mesangial cell proliferation is mediated by the PGE2 receptor subtype EP4. Activation of the EP1 receptor subtype stimulates DNA synthesis in mesangial cells [56]. The formation of PGE2 in mesangial cells is increased by the cytokines interleukin-1-beta and PDGF [57]. The vasoconstrictors endothelin-1 and angiotensin II also stimulate the synthesis and release of PGE2. On the other hand PGE2 prevents the contractile effect of the vasoconstrictor angiotensin II in a dose-dependent manner [58]. Furthermore, PGE2 is able to increase the serum m-RNA level of matrix-metalloproteinase 2 (MMP-2) [59]. In cultured rat mesangial cells MMP-2 is important for the degradation of collagen type IV. PGE2 not only stimulates the degradation of extracellular matrix proteins but also reduces mRNA levels of procollagen I and III and the secretion of collagen I, III, IV and fibronectin [60]. Jaffer et al. demonstrated that PGE2 stimulates migration of mesangial cells which may play a role in some forms of glomerulonephritis [61].

The effect of prostacyclin (prostaglandin I2, PG12) on mesangial cells was examined using beraprost-sodium, a prostacyclin analog. Beraprost-sodium inhibits rat mesangial cell proliferation. This effect is caused by a cyclic AMP dependent induction of mitogen-activated protein kinase phosphatase (MKP-1) which inhibits the MAP kinase pathway [62]. The function of prostacyclin is mediated by a prostacyclin receptor (IP receptor) which is expressed by mesangial cells [63]. In a rat model of crescentic glomerulonephritis, infusion of PG12 led to a decrease in proteinuria, fibrinogen deposition and crescent formation [64]. This is caused by an inhibition of intraglomerular coagulation and by downregulation of intercellular adhesion molecule-1 (ICAM-1) expression which decreases leukocyte infiltration.

5. Adenosine

Adenosine is a unique signaling molecule because it causes vasodilation in most of the blood vessels (e.g. peripheral arteries, coronary arteries) while it is a vasoconstrictor in afferent glomerular arterioli of the kidney [65]. Formation of adenosine is the result of nucleotide breakdown and occurs in tissue hypoxia and ischemia [66]. The cellular effects of adenosine are mediated by adenosine receptors which are members of the G-protein coupled receptor family [67]. The family of adenosine receptors includes four members: A1, A2a, A2b and A3 receptors. Adenosine A1 receptors have been shown to be coupled to activation of phospholipase C and increase of intracellular Ca2+, while the other three subtypes have been found to
couple to cyclic AMP formation. As a result of these different signaling pathways, adenosine A1 receptors were found to mediate vasoconstriction while the other three receptors cause vasodilatation. Accordingly, A1-receptors have been found in afferent arteriolar vessels of the rat kidney while the A2 receptor is the predominant receptor of the arterial vasculature of the periphery and coronary arteries. A3 receptors have been detected in the brain and appear to play role in neurotransmission [68]. In addition, adenosine has been shown to mediate tubuloglomerular feedback in the kidney and thereby regulates glomerular ultrafiltration [69]. Since mesangial cells are thought to play a central role in the pathogenesis of glomerular disease several authors examined the regulation of mesangial cell function by adenosine (Table 1). Cultured mesangial cells were found to express both A1 and A2-receptors. In this cell type, A1-receptors stimulated Ca ++ uptake, while A2-receptors enhanced cyclic AMP accumulation [70]. In cell culture, adenosine caused mesangial cell proliferation and contraction by an A1-type receptor [71,72]. Not many data are available on the regulation of cultured preglomerular smooth muscle cells by extracellular adenosine. In cultures of these cells, extracellular adenosine levels and intracellular cAMP have been shown to be dependent on the genetic background (normotensive vs. spontaneously hypertensive rats) and extracellular stimuli, e.g. isoproterenol [73,74]. Because rat glomerular microcirculation was shown to be regulated by renal adenosine-1 receptors, several therapeutic approaches have been developed to utilize adenosine agonists and antagonists in therapy of kidney disease [75]. Recently, highly selective adenosine-A1-receptor antagonists have been demonstrated to exert natriuretic and diuretic actions in experimental animals [76,77]. Currently the diuretic effect of A1-receptor blockers is examined in congestive heart failure in humans. In a study by Poelstra et al., injection of the adenosine analogue 2-chloro-adenosine ameliorated the course of anti-Thy 1 glomerulonephritis and reduced proteinuria in the rat indicating a beneficial effect mediated by adenosine [78]. According to the current view, reduction in proteinuria may be due to altered glomerular hemodynamics and increased afferent glomerular vasoconstriction caused by increased extracellular adenosine. It is possible that adenosine agonists and antagonists may be useful drugs for therapy of kidney disease.

6. Conclusion

Glomerular cells, particularly mesangial cells, are exposed to signals from numerous vasoconstrictor and vasodilator hormones which are present in the kidney. Under physiological conditions, a fine balance between these signals is maintained resulting in the normal homeostasis of the glomerulus. In chronic glomerular disease, glomerular hypertension due to increased action of vasoconstrictors such as angiotensin II is found to play a detrimental role accelerating the process of deterioration of renal function. Therapeutic intervention in the processes leading to end-stage glomerular disease is possible at several levels. First, antiproliferative vasodilatory factors such as ANP, nitric oxide or PGE2 may block mesangial cell proliferation and thereby exert a beneficial effect in mesangial proliferative glomerulonephritis. Secondly, vasoconstriction of efferent glomerular arterioles may be reduced by vasodilatory signaling molecules which ultimately reduce glomerular hypertension and progression to glomerular sclerosis. Thirdly, vasoconstriction of afferent glomerular arterioles by adenosine may functionally counteract vasoconstriction of efferent glomerular arterioles caused by angiotensin II and thereby ameliorates glomerular hypertension. Based on this concept, potential beneficial effects of natriuretic peptides, nitric oxide, prostaglandines and adenosine in glomerular disease of the kidney should be examined more extensively to develop new therapeutic approaches in these diseases.

Recently, vasopeptidase inhibitors such as omapatrilat were used to increase plasma levels of natriuretic peptides to utilize potential beneficial effects of these molecules [79,80]. In an experimental model of heart failure, omapatrilat increased renal plasma flow and glomerular filtration rate after both acute and repeated dosing [80]. Moreover, in a recent clinical trial in patients with heart failure the vasopeptidase inhibitor omapatrilat appeared to be more favorable than the angiotensin-converting enzyme inhibitor lisinopril [81]. These studies indicate that increasing the activity of vasodilatory factors such as natriuretic peptides may be a promising approach for therapy of glomerular disease and chronic renal failure as well as heart failure [82]. However, the use of vasodilatory molecules may be limited by side-effects such as hypotension. Additional clinical trials in patients with progressive renal disease treated with vasopeptidase inhibitors as well as the development of new drugs which enhance activity of vasodilatory factors will ultimately clarify the potential use of these substances as renal protective agents.

References


[35] Kunz D, Walker G, Pfeifschrifer J. Transforming growth factor-beta...


