Signal Transduction in Mesangial Cells

Paolo Menè,1 Giulio Alberto Cinotti, and Francesco Pugliese

ABSTRACT
Phenotype, growth, and functional characteristics of glomerular mesangial "myofibroblasts" are under the control of multiple hormones, vasoactive agents, autacoids, and cytokines. Several parallel signal transduction pathways couple receptor occupancy with functional changes, including phospholipases C, A2, and D breakdown of membrane phospholipids, and adenylate/guanylate cyclase activation. Changes of cytosolic ion concentrations, cyclic nucleotide accumulation, and eicosanoid biosynthesis are currently interpreted as intracellular signals for protein kinase activation. Phosphorylation of multiple substrates by serine/threonine kinases C, A, and G or by tyrosine kinases directly coupled to receptors, is a final step in cell activation. Cross-talk between signal transduction pathways, along with the release of eicosanoids and cytokines acting as intercellular mediators, provides the potential for interactive regulation of glomerular cell functions.

Key Words: G protein, protein kinases, adenylate cyclase, guanylate cyclase, phospholipases

Renal glomerular mesangial cells structurally and functionally resemble perivascular smooth muscle cells (1,2). Various mesangial functions, described elsewhere in this issue, are under the control of neighboring glomerular cells, infiltrating bone marrow-derived cells, circulating hormones, or local hemodynamics via a complex array of plasma membrane sensor/receptors and intracellular signal transduction mechanisms. Most known mesangial pathways of signal transduction obey the basic sequence depicted in Figure 1. After occupancy or stimulation of a cell surface receptor, conformational changes of a strictly associated GTP-binding protein, or G protein, activate a membrane-linked or cytosolic enzyme to release a diffusible molecule from a phosphorylated substrate. These signal molecules act as intracellular "second messengers" to elicit conformational changes in specific protein kinases, which in turn phosphorylate several substrates to yield the required functional responses. The best known such signal transduction pathways are the "classic" adenylate cyclase, guanylate cyclase, and phospholipase C/A2 systems. Several agents can bypass one or more of these steps, as in the case of stimuli of soluble guanylate cyclase, which can permeate and thus bypass the plasma membrane, or of tyrosine kinase(s), usually directly coupled to the surface receptors. Other compounds can be internalized and hence act upon intracellular or nuclear receptors, as may be the case for 1,25(OH)2D3.

Extensive evidence that certain mesangial cell functions, such as contraction and proliferation, are controlled, among other factors, by these signaling systems has been gathered during the past decade. Current knowledge on how extracellular molecules or physical changes convey information to the intracellular environment will be briefly reviewed, in light of the pivotal role of the mesangium in renal pathophysiology.

ADENYLATE CYCLASE

Consistent with other contractile cell types, the intracellular formation of cyclic nucleotides follows exposure of mesangial cells to several vasodilator agents (2). Adenylate cyclase is responsible for the accumulation of cAMP upon the binding of several ligands, listed in Table 1, to surface receptors coupled to G proteins (Figure 1). Typical stimuli of mesangial adenylate cyclase include catecholamines and vasodilator prostaglandins (PG) (3–6). The release of cAMP, whose levels are regulated by formation, extrusion towards the extracellular space, and degradation by phosphodiesterases, controls the activity of a specific kinase, termed protein kinase A (PKA) (7). PKA is in turn responsible for the final cellular effects, such as the inhibitory phosphorylation of myosin light chain kinase, thus blocking contraction. Recently, cAMP has been shown to signal the anti-mitogenic effects of certain PG on mesangial cell cultures (7,8). The intracellular site of action of cAMP or PKA is unknown, although putative cAMP response elements have been described on several early growth response genes of eukaryotic cells (9).
The soluble form of guanylate cyclase has recently been the object of extensive investigation, in view of the potential effects of endothelial-derived relaxing factors on the contractile mesangium. Coculture systems combining endothelial cells from various organs and mesangial cells have demonstrated that the latter are a major target for nitric oxide (NO) and various nitrates, currently believed to be among the major mediators of endothelium-dependent smooth muscle relaxation (18,19). cGMP stimulation in this case results from the permeation of the plasma membrane by nitrates, which directly activate soluble guanylate cyclase. Similar to previous observations with ANP, NO inhibits the proliferation of mesangial cells in culture, confirming the inhibitory effects of cGMP on DNA synthesis/gene expression, and suggesting a possible “tonic” regulatory role of the glomerular endothelium on the growth of the underlying mesangium. Nitrates also appear to modulate [Ca^{2+}]_i, in agreement with earlier ANP data (20). However, a recent report in fibroblasts devoid of guanylate cyclase activity points to cGMP-independent pathways of [Ca^{2+}]_i modulation (20). This could explain the discrepancy between cGMP stimulation and the failure to modify [Ca^{2+}]_i, occasionally reported by some investigators (14,15), and could indicate other, as yet unknown, signal transduction mechanisms possibly activated by stimuli of guanylate cyclase. The functional significance of cGMP stimulation by cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF) is not known (21). An intriguing possibility is that soluble guanylate cyclase becomes activated by endogenous NO arising from the cytokine induction of a mesangial NO synthase. Abrogation of cGMP responses to IL-1 and TNF by growth factors or specific inhibitors of NO synthase is consistent with this recent proposal (22).

**PHOSPHOLIPASE C**

A number of vasoconstrictors and growth factors bind to mesangial receptors coupled to phospholipase C (Table 2). After the original reports of phosphoinositide breakdown upon stimulation of the cells with angiotensin II (ANG II) and arginine vasopressin (23), various studies have analyzed the patterns of inositol phosphate (InsP) and diacylglycerol (DAG) in

![GUANYLATE CYCLASE](image)

**Table 1. Stimuli of mesangial cell cyclic nucleotides**

<table>
<thead>
<tr>
<th>Stimuli of mesangial cell cyclic nucleotides</th>
<th>AMP</th>
<th>Dopamine</th>
<th>Histamine</th>
<th>PGE2 and PG12</th>
<th>Forskolin</th>
<th>ANP</th>
<th>GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol (epinephrine, norepinephrine)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE2 and PG12</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>GMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Endothelial-Derived Relaxing Factor (NO, Nitrates)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1, TNF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Stimuli of mesangial cell cyclic nucleotides**

<table>
<thead>
<tr>
<th>Stimuli of mesangial cell cyclic nucleotides</th>
<th>AMP</th>
<th>Dopamine</th>
<th>Histamine</th>
<th>PGE2 and PG12</th>
<th>Forskolin</th>
<th>ANP</th>
<th>GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol (epinephrine, norepinephrine)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE2 and PG12</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>GMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Endothelial-Derived Relaxing Factor (NO, Nitrates)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1, TNF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1. Major pathways of transmembrane signal transduction in cultured mesangial cells. Abbreviations: A, agonist; R, receptor; G, GTP-binding protein; PLC, phospholipase C; PLAs, phospholipase A2; AC, adenylate cyclase; GC, guanylate cyclase; PC, PE, PIP2, phosphatidylcholine, phosphatidylinositolamine, and phosphatidylinositolbis-phosphate, respectively; PI, phosphoinositides; IP3, inositol(1,4,5)-trisphosphate; I/C, intracellular; E/C, extracellular; TK, tyrosine kinase.**
response to vasoconstrictors (24). These events result in \([\text{Ca}^{2+}]_i\) elevation and protein kinase C (PKC) translocation to the plasma membrane, respectively (Figures 1 and 2). Radioisotopic studies and optical techniques employing ion-sensitive fluoroprobes confirmed earlier observations on the time course and cellular byproducts of phospholipase C activation in other mesenchymally derived cells (2, 24). We have focused our attention on arachidonate (AA) metabolites acting upon phospholipase C, because cyclooxygenase and, to a lesser extent, lipooxygenase and cytochrome P-450 products can arise from activated mesangial cells and thus act as autocrine factors. Thromboxane A2 and PGF2α are the major cyclooxygenase metabolites of AA acting on human and rat mesangial cell phospholipase C, respectively, whereas leukotriene D4 is the most prominent such lipooxygenase derivative (25, 26). Similar to ANG II and other vasoconstrictors, all of these compounds discharge intracellular \([\text{Ca}^{2+}]_i\) stores, an Ins(1,4,5)-P3-mediated process, resulting in a sharp and transient elevation of \([\text{Ca}^{2+}]_i\) (Figure 2). The contribution of \([\text{Ca}^{2+}]_i\) entry through plasma membrane channels appears of lesser importance, whereas its extrusion via \([\text{Ca}^{2+}]_i\) pumps, outward leak paths, and Na+/Ca2+ exchange concurs to rapidly restoring prestimulation \([\text{Ca}^{2+}]_i\) levels (27). Nevertheless, these rapid \([\text{Ca}^{2+}]_i\) changes signal for a variety of intracellular events, including phospholipase A2 activation, opening of membrane Na+ and Cl− conductances with resulting membrane depolarization, and H+ extrusion (28, 29). Enhanced Cl−/HCO3− exchange readily offsets activation of Na+/H+ exchange, thus preserving intracellular pH (30). \([\text{Ca}^{2+}]_i\)-sensing cytosolic or cytoskeletal proteins, including calmodulin and caldesmon, undoubtedly play a role in transducing \([\text{Ca}^{2+}]_i\) changes into functional responses, such as activating phosphorylation of myosin light chain kinase, resulting in cell contraction. However, few or no data are available for mesangial cells in this area.

The functional consequences of phospholipase C activation cannot be entirely attributed to changes of \([\text{Ca}^{2+}]_i\). Several lines of evidence demonstrate in fact that serine/threonine kinase C by itself can modify mesangial cell structure and functions. The use of phorbol esters and a wide variety of kinase inhibitors confirmed that PG synthesis, contraction, and mitogenesis can be induced solely by PKC activation, in the absence of apparent changes of \([\text{Ca}^{2+}]_i\) (31, 32). Stimulation of Na+/H+ exchange with resulting cytosolic alkalinization in the absence of HCO3− is a PKC-dependent process (29). Even tyrosine phosphorylation promoted by certain vasoconstrictors may be at least partly dependent upon PKC activation (33). Recently, cloning and sequencing of PKC has resulted in the availability of antibodies to screen mesangial cells for isoforms of the enzyme. Preliminary data presented at this meeting suggest that the isoforms α and ε are the major PKC subspecies in mesangial cells and that they control feedback inhibition of phospholipase C and phospholipase A2 activity, respectively (34).

**PHOSPHOLIPASE A2 AND D**

A link between ligand-induced activation of different phospholipases is quite clear in mesangial cells, on the basis of an analysis of the water-soluble and lipid components of stimulated cells. Activation of phospholipase C is almost constantly accompanied by AA mobilization and phosphatidic acid (PA) accumulation (24). Pathways for the separation of these components from the parent membrane phospholipid molecule include phospholipase D, which selectively cleaves PA, and phospholipase A2/DAG lipase, which can release AA from phospholipid or DAG, respectively (35, 36). Phospholipase A2 is potently activated both in vitro and in vivo by \([\text{Ca}^{2+}]_i\). Additionally, PA can be reformed by the activity of DAG kinase upon DAG. The use of specific inhibitors has provided evidence for the presence of all of these enzymes in mesangial cells, as well as for their activation by vasoconstrictor agents. The availability of DAG upon phospholipid metabolism by phospholipase C and the rapid changes of \([\text{Ca}^{2+}]_i\), resulting from Ins(1,4,5)-P3 formation promote the activity of such lipid breakdown pathways. Recently, evidence for a separate coupling of vasoconstrictor receptors with phospholipases A2 and C has surfaced, based on differential inhibition of PG synthesis and InsP accumulation by the GTP-binding protein, ADP-ribosylating agent,
pertussis toxin, and by PKC isoenzyme activation/inhibition with phorbol esters (34,37). The presence of a soluble form of phospholipase A₂ has also been proposed in agonist-stimulated cells, with potential implications as an extracellular signaling apparatus (38). The metabolism of AA bridges, in fact, intracellular second messengers with extracellular signaling. Most eicosanoids, i.e., 20-carbon-atom molecules derived from AA cyclooxygenation or lipoxygenation, recognize specific receptors on the surface of mesangial cells that are coupled either to phospholipase C/A₂ or adenylate cyclase. Thus, PG, leukotrienes, and hydro(peroxy)eicosatetraenoic acid (HETE, HPETE) may act as autocrine or paracrine factors, binding to the same cells that release them or to contiguous cells. In this view, these compounds serve as an extracellular branch of the phospholipase(s) intracellular signaling system. The issue of whether the extracellular concentration of such agents ever reaches levels compatible with sufficient receptor occupancy needs to be addressed. Such interaction might in fact occur at the level of single cells or even limited areas of the plasma membrane.

Recently, another potential role of eicosanoids has
been identified in cultured rat mesangial cells. Cyto-
chrome P-450 metabolites of AA have been shown to
amplify the [Ca\(^{2+}\)]\(_i\) response to phospholipase C stim-
uli and to mediate, at least in part, the mitogenic
response to serum and growth factors (39,40). These
studies demonstrated that the blockade of the P-450
and/or lipoxygenase pathways interferes with the
discharge of intracellular [Ca\(^{2+}\)]\(_i\) stores by InsP\(_3\)
and proliferation. According to the model proposed by
those investigators, 14,15-epoxyeicosatrienoic acid
and 15-HPETE act as Ca\(^{2+}\)-mobilizing agents, access-
ing pools of intracellular Ca\(^{2+}\) that are additive to
those gated by InsP\(_3\). Therefore, at least two addi-
tional intracellular messengers would be involved in
the mobilization of Ca\(^{2+}\) in this cell type, in addition
to the “classic” InsP\(_3\) mechanism. Whether this effect
of AA metabolites directly translates into the stimu-
lation of DNA synthesis or whether other interactions
occur between these eicosanoids and growth re-
sponse genes is still unknown. Clearly, these studies
indicate that AA metabolites can act both extracel-
lularly and intracellularly to mediate and/or amplify
responses to phospholipase C agonists.

OTHER PROTEIN KINASES

Tyrosine kinase is an integral part of the receptors
for most peptide growth factors, including insulin
and certain agents that also stimulate phospholipase
C, such as bombesin and platelet-derived growth fac-
tor. Tyrosine phosphorylation of multiple cellular
substrates is therefore an early consequence of the
mitogenic stimulation of mesangial cells (41). Re-
cently, a link between the phospholipase C system
and tyrosine kinase activity has been identified in
mesangial cells. Agents such as arginine vasopres-
sin, ANG II, and endothelin enhance tyrosine kinase
activity via both PKC-dependent and PKC-independent
pathways, as indicated by studies with phorbol
esters and PKC inhibitors (33). Tyrosine kinase acti-
vation may well be the central event that explains
the mitogenic properties of these vasoconstrictors.

Multiple kinases, in addition or alternative to the
well-known serine/threonine kinases C and A and
tyrosine kinase, may be involved in signal transduc-
tion for compounds such as IL-1 or TNF (Table 3). At
variance with “conventional” agonists of mesangial
cells, these cytokines appear to employ parallel signal
transduction pathways, including cGMP/PKG, non-
phosphatidylinositolbisphosphate-specific phospho-
lipase C, and direct activation of other kinases
(21,42,43).

In conclusion, the recent acquisitions in the field
of transmembrane signal transduction have greatly
expanded our understanding of mesangial cell patho-
physiology. Given the key role played by the mesan-
gium in normal ultrafiltration and its frequent in-

<table>
<thead>
<tr>
<th>TABLE 3. Stimuli of mesangial cell protein kinases (other than A, G, and C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>Fibroblast growth factor</td>
</tr>
<tr>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>Insulin, insulin-like Growth Factor (1)</td>
</tr>
<tr>
<td>IL-1, IL-6</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
</tr>
</tbody>
</table>

volvement in glomerular disease, the identification
of biochemical pathways that convey information to
intracellular effector organs is important in planning
therapeutic approaches. The discovery of a variety of
diffusible mediators that initiate and maintain mes-
angial inflammation provides an ideal target for
pharmacological intervention on the initial lesion
and, later, reparative sclerosis. Recent in vivo re-
search designed on the basis of experimental evi-
dence for the mesangial actions of vasoactive agents
and peptide growth factors provides an example of
the potential implications of this type of studies
(44,45).

ACKNOWLEDGMENTS

This work was supported in part by grants from the Ministero
dell'Universit\'e e della Ricerca Scientifica of Italy (quote 40–60%).

REFERENCES

1. Latta H. Ultrastructure of the glomerulus and
juxtalamellar apparatus. In: Orloff J, Berliner
RW, Geiger SR, eds. Handbook of Physiology.

2. Men\'e P, Simonson MS, Dunn MJ: Physiology of the
mesangial cell. Physiol Rev 1989;69:1347–
1424.

3. Kreisberg JI, Venkatachalam MA, Patel PY:
Cyclic AMP-associated shape change in mesan-
gial cells and its reversal by prostaglandin \(E_2\).

4. Barnett R, Singhal PC, Scharschmidt LA,
Schlondorff D: Dopamine attenuates the con-
tractile response to angiotensin II in isolated rat
glomeruli and cultured mesangial cells. Circ Res

5. Friedlander G, Chandel D, Sraer J, Bens M,
Ardaillou R: PGE\(_2\) binding sites and PG-stimu-
lated cyclic AMP accumulation in rat isolated
glomeruli and glomerular cultured cells. Mol Cell

6. Men\'e P, Dunn MJ: Eicosanoids and control of
mesangial cell contraction. Circ Res 1988;62:
916–925.

7. Men\'e P, Dunn MJ: Prostaglandins and rat glo-
merular mesangial cell proliferation. Kidney Int

8. Men\'e P, Abboud HE, Dunn MJ: Regulation of
human mesangial cell growth in culture by
thromboxane \(A_2\) and prostacyclin. Kidney Int

9. Sukhatme VP: Early transcriptional events in

Volume 2 • Supplement 2 • 1992


