Cell Surface Receptors and Ectoenzymes in Mesangial Cells

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ABSTRACT

Mesangial cells possess a variety of receptors for hormones and autacoids. They are also equipped with ectoenzymes whose function may be to control the availability of autacoids and hormones at their receptor sites. Several examples are considered. Receptors for angiotensin II (All) are present both on murine and human mesangial cells. One single group of receptors has been demonstrated in each of these preparations. Mesangial cell All receptors are linked to phospholipase C via a G protein. They belong to the AT₁ subtype because ([125I]All is displaced from its binding sites preferentially by AT₁ antagonists such as DUP 753 and EXP 3174, whereas AT₂ antagonists are much less potent. AT₁ antagonists suppress the biological effects of All in mesangial cells. AT₂ antagonists are much less potent. AT₁ antagonists suppress the biological effects of All in mesangial cells, including the stimulation of intracellular calcium concentration and the increase of prostaglandin E₂ and cAMP. Various other cAMP-stimulating guanylate cyclase activity and clearance (C) factor, but the distribution between B receptors with calcium concentration and the increase of prostaglandin E₂ and cAMP is marked in rat mesangial cells by interleukin-1, whose effect is mediated in part by prostaglandin E₂, and cAMP. Various other cAMP-stimulating agents also induce 5'-nucleotidase expression in rat mesangial cells. Ectopeptidases are present in all glomerular cell types but essentially in epithelial cells. Mesangial cells exhibit aminopeptidase N activity. Expression of this enzyme is induced by phorbol esters via a protein kinase C-dependent mechanism. These results indicate that, in addition to membrane receptors, ectoenzymes have to be considered in the control of mesangial cell functions.

Key Words: Mesangial cell, angiotensin II, atrial natriuretic factor, 5′-nucleotidase, aminopeptidase

Mesangial cells play a key role in glomerular physiology for the following reasons: (1) their degree of contraction directly controls the surface of the glomerular capillaries available for filtration; (2) they are the source of a variety of autacoids including metabolites of arachidonic acid, cytokines, reactive oxygen species, nitric oxide, endothelin, and growth factors, which act in an autocrine and paracrine fashion on the glomerular functions; (3) they can take up and metabolize foreign agents and immune complexes (for a review, see reference 1). Mesangial cell functions are regulated by hormones and autacoids. Enzymes localized at the surface of the glomerular cells (ectoenzymes) mediate the availability of these hormones and autacoids at their receptor sites through different mechanisms: (1) they generate autacoids by hydrolysis of extracellular substrates. For example, ecto-5′-nucleotidase controls the production of adenosine from AMP. (2) They transform proforms of hormones or autacoids into their final active forms. This is the case of the converting enzymes of angiotensin II (All) and endothelin. (3) They degrade hormones or autacoids into inactive peptides. For example, neutral endopeptidase splits the ring of atrial natriuretic factor (ANF) necessary for its biological activity. Catabolism may also result in metabolites of lesser activity. In this manner, aminopeptidase A controls the production of Des Asp¹ All from All. (4) They may play important roles in signal transduction at the cell membrane and in the binding to and degradation of the extracellular matrix. This article is not a review of all mesangial cell receptors and ectoenzymes. Our aim is to provide examples based on studies from our laboratory. In particular, we will consider the receptors for the two main hormones regulating GFR, i.e., All and ANF. We will also examine the roles of ecto-5′-nucleotidase as the main enzyme involved in the formation of adenosine and of ectopeptidases as catabolizing agents of the peptidic hormones.
MESANGIAL CELL RECEPTORS FOR ALL

The receptor sites for All in the glomeruli are mainly located on the mesangial cells. Osborne et al. (2), by using autoradiographic techniques, showed that tritiated All injected into the renal artery of rats was concentrated by the mesangial cells. Autoradiographic studies performed with \[^{125}I\]All confirmed these results (3). With rat cultured mesangial cells, we demonstrated the presence of high-affinity binding sites for All (4). The \(K_D\) value and the number of sites were 2.8 nM and 44 fmol/mg of protein (about 20,000 receptor sites per cell), respectively. There was only one group of receptor sites. Equilibrium of binding occurred after 30 to 40 min of incubation at 22°C. Binding was maximum in the presence of calcium (1 to 5 mM) in the incubation medium. The physiological relevance of these receptors was based on their linkage to cell contractility. Indeed, we observed in this early study changes in the length or shape of the mesangial cells within a few minutes after incubation with 1 nM All. Interestingly, the myosin distribution in the contracting mesangial cells was quite different from that observed in the noncontracting ones. The percentage of contractile cells increased progressively up to 32% with the concentration of All and reached a steady-state value above 10 nM. Extracellular calcium was necessary both for \[^{125}I\]All binding and cell contraction. Sar\(^1\),ala\(^8\) All, a peptide antagonist of All, inhibited both events. Contraction of rat mesangial cells by All has been extensively studied in the literature. It corresponds to a maximum decrease of 10 to 15% in the surface area of the cells (5,6).

More recently, we have studied the binding sites of All in human mesangial cells and have demonstrated that they belong to the AT\(_1\) subtype (7). \[^{125}I\]sar\(^1\),ala\(^8\) All was used as tracer because it is more resistant to degradation than was \[^{125}I\]All. \[^{125}I\]sar\(^1\),ala\(^8\) All bound to human mesangial cells in a specific saturable manner. The \(K_D\) value was 0.37 nM, but the density of receptor sites was low (7 fmol/mg or 3,200 receptor sites per cell) when compared with that observed in human whole glomeruli. A plateau of binding was reached after 30 min of incubation at 22°C. Binding was also maximum in the presence of calcium (1 to 5 mM) in the extracellular medium. Competitive inhibition experiments were performed with nonpeptide and peptide antagonists of All. Sar\(^1\),ala\(^8\) All and EXP 3174, an AT\(_1\) receptor antagonist, exhibited similar potencies with IC\(_{50}\) values of 0.35 and 0.28 nM, respectively. DUP 753, another AT\(_1\) antagonist, considered as the precursor of EXP 3174, was slightly less potent than its metabolite with an IC\(_{50}\) of 6.2 nM. In contrast, the AT\(_2\) nonpeptide antagonist, PD 123177, displaced \[^{125}I\]sar\(^1\),ala\(^8\) All only at high concentrations. The AT\(_2\) peptide antagonist CGP 42112 A exhibited an intermediary potency between DUP 753 and PD 123177. Another reason to classify these All receptors as AT\(_2\) is their sensitivity to di-thiothreitol, shown by the inhibition of binding of \[^{125}I\]All in the presence of this drug. Saturation binding experiments were also performed in the presence of a fixed concentration of DUP 753 (5 nM) or of EXP 3174 (1 nM). In both cases, the \(K_D\) values (0.33 and 0.40 nM, respectively) did not differ from that observed under control conditions, whereas the number of sites was markedly diminished. This suggests that DUP 753 and EXP 3174 behave as noncompetitive antagonists in this preparation. As in rat mesangial cells, we found that All produced a decrease in the cell surface area of human mesangial cells at the concentration of 1 nM. The percentage of contracting cells was 40 to 60%. Changes were visible at 2 to 5 min and progressed up to 15 min. Semiquantitative evaluation of cell contraction was performed by morphometry of cell area. There was a 18% decrease after 15 min of incubation of the cells with 1 nM All (8).

Mesangial cell contractility represents the main short-term effect of All. After binding to its receptor, All stimulates phospholipase C activity (Figure 1). Transmembrane signaling involves a GTP-binding protein (G protein) that can be inactivated by ADP ribosylation catalyzed by pertussis toxin (9). A supplementary proof for the role of a G protein is the marked inhibitory effect of GTP or its stable analogs such as Gpp(NH)p on the binding of \[^{125}I\]All to its receptors. Phospholipase C activation generates from phosphatidylinositol-4,5-bisphosphate two second messengers, 1,4,5-inositol triphosphate (IP\(_3\)) and diacylglycerol (DAG). IP\(_3\) is not the only inositol phosphate produced in mesangial cells exposed to All. Shin et al. (10) have also detected inositol-1,3,4,5-tetrakisphosphate formed by the phosphorylation of IP\(_3\) and 1,3,4-trisphosphate resulting from a subsequent dephosphorylation. IP\(_3\) and probably the other inositol phosphates induce the release of calcium from its intracellular stores in the endoplasmic reticulum. This is the main mechanism for the All-dependent increase of cytosolic calcium concentration (Ca\(^{2+}\)). The peak of Ca\(^{2+}\) occurs immediately after exposure of mesangial cells to All. It is brief with a return of Ca\(^{2+}\) to its basal level within 1 min (Figure 2). This effect is modulated by protein kinase C (PKC) activity. The stimulation of PKC decreases the amplitude of the Ca\(^{2+}\) peak, whereas the inhibition of PKC by a long-term pretreatment with phorbol esters potentiates the effect of All on IP\(_3\) formation, calcium mobilization, and the subsequent contraction of mesangial cells (11). It has been also suggested that All stimulated the entry of extracellular calcium in the cell via receptor-operated calcium channels because the Ca\(^{2+}\) peak is attenuated after treatment by dithiodyridines (12). The contraction of mesangial cells
directly depends on Ca$^{2+}$, which activates myosin light chain kinase, resulting in myosin phosphorylation. Responses to AII seem to decline with time in culture, suggesting loss of receptors or functional uncoupling of binding sites from phospholipase C. Simultaneously with the effect on Ca$^{2+}$, AII depolarizes mesangial cells by activating chloride (Cl$^-$) channels. This activation is mediated by the increase in Ca$^{2+}$ (13). Conversely, the response of mesangial cells to AII depends on the extracellular concentration of Cl$^-$.

The percentage of contracting cells and the amplitude of the Ca$^{2+}$ peak are diminished in the absence of extracellular Cl$^-$. The mechanism of this effect seems to be an increase in prostaglandin E$_2$ (PGE$_2$) production. Such a sequence of events might occur in the glomerular response to an increased Cl$^-$ absorption at the macula densa, signal starting the tubuloglomerular feedback.

In addition to calcium mobilization, the second immediate effect of AII is the increase of PG production by the stimulation of phospholipase activity. AII produces within 2 min a twofold to threefold increase of
the synthesis of 6-keto-PGF\textsubscript{1\alpha} and PGE\textsubscript{2} in human mesangial cells (8). The threshold for stimulation occurs at 10 pM. The mechanism of this effect is complex. All activates phospholipase A\textsubscript{2} via an increase in Ca\textsuperscript{2+} and also via PKC-mediated inhibition of lipocortin. Furthermore, the hydrolysis of DAG by diglyceride lipase generates arachidonic acid. Craven et al. (14) have reexamined the role of PKC in All-dependent PGE\textsubscript{2} synthesis by rat glomeruli. For these authors, PKC is a negative modulator of All effects on PGE\textsubscript{2} because the inhibition of PKC was stimulatory, whereas its stimulation inhibited All-dependent PGE\textsubscript{2} synthesis. PGE\textsubscript{2} is the main PG produced in rat mesangial cells, whereas PGI\textsubscript{2} is predominant in human mesangial cells. Both PG stimulate cAMP formation and thereby limit the effect of All on mesangial cell contraction. Foidart and Mahieu (15) showed that the percentage of cells contracting in response to All was markedly increased after pretreatment by cyclooxygenase inhibitors, whereas it was decreased in the presence of exogenous PGE\textsubscript{2}. It has been also proposed in other systems that All inhibits adenylate cyclase activity via a G\textsubscript{i} protein. Such an effect of All has never been demonstrated in glomeruli and mesangial cells (16).

All produces long-term effects on mesangial cells. Treatment by All results essentially in cell hypertrophy with an increase of protein synthesis (17). All may also behave like a mitogen, but the latter effect is not observed permanently. It has been proposed that All acted indirectly via the induction of the synthesis of platelet-derived growth factor, which is a potent mitogen of mesangial cells (18). The effect of All on cell growth is self limited by the increase in the synthesis of PGE\textsubscript{2}, which, via cAMP, inhibits the proliferation of mesangial cells (19). The long-term effects of All are mediated by the phospholipase C pathway. The exposure of vascular smooth muscle cells to All results in early mitogenic events such as an increase in pH\textsubscript{i} and Ca\textsuperscript{2+}, and stimulation of the transcription of the proto-oncogenes c-fos, c-jun, and c-myc. These effects of All on cell hypertrophy and cell growth can result in an increase of the peripheral vascular resistance and can also be a factor of the development of glomerular sclerosis. The latter hypothesis is reinforced by the beneficial effect of a long-term treatment by a converting enzyme inhibitor on the progression of glomerular injury in rats with experimental models of renal disease (20).

We have recently demonstrated that the functional effects of All on mesangial cells were all dependent on AT\textsubscript{1} receptors (7). Indeed, DUP 753 and EXP 3174 markedly inhibited the All-stimulated increase of PGE\textsubscript{2} production, Ca\textsuperscript{2+}, and \textsuperscript{3}Hleucine incorporation into cell proteins. The concentrations needed for these effects were in the same range as those inhibiting \textsuperscript{125}I\textsuperscript{a}sar\textsuperscript{1},ala\textsuperscript{6} All binding to mesangial cells. The AT\textsubscript{1} receptor has recently been cloned (21,22). The derived protein structure consists of 359 amino acids. Binding studies have been performed in transfected COS-7 cells expressing AT\textsubscript{1}. The $K_{D}$ value observed (0.68 nM) is very close to that found in human mesangial cells (0.37 nM). The AT\textsubscript{1} receptor exhibits the seven transmembrane domains characteristic of the G-protein-coupled superfamily of receptors. The hypothesis that the AT\textsubscript{1} receptor corresponded to the mas oncogene product has now been rejected.

MESANGIAL CELL RECEPTORS FOR ANF

Both murine and human mesangial cells possess ANF receptors. However, the distribution of the two subtypes of ANF receptors (B and C) is not identical in mesangial cells of both species. B receptors contain guanylate cyclase in the cytoplasmic domain of their molecules and mediate the ANF-induced increase in cGMP. C receptors have an important role in the removal of ANF from the circulation via its internalization (Figure 3). Ballermann et al. (23) reported a single binding site for \textsuperscript{125}I ANF on rat mesangial cells with a $K_{D}$ value of 0.5 nM. ANF stimulated cGMP generation with a threshold of 1 to 10 nM. In contrast, negligible binding and cGMP accumulation were found in glomerular epithelial cells. Appel et al. (24) confirmed that ANF dose dependently raised cGMP in mesangial cells and also showed that ANF inhibited All-induced rat mesangial cell contraction as well as IP\textsubscript{3} accumulation and Ca\textsuperscript{2+}; response. Binding studies with \textsuperscript{125}I ANF showed a high density of total ANF receptors in cultured rat mesangial cells with a $K_{D}$ of 77 pM. Both types of receptors with a similar high affinity for native ANF-(1-28) were present. C receptors representing approximately 30% of the total ANF receptor population. The part of C receptors diminished in cells that were studied in the latest passages (25). In contrast, Bianchi et al. (3) and Koseki et al. (26), by autoradiographic techniques, found that radiolabeled ANF was predominantly distributed in glomerular epithelial cells on rat renal sections. We showed in an early report (27) that ANF markedly stimulated cGMP synthesis in human cultured glomerular epithelial cells, whereas its effect on mesangial cells was only minor. More recently, we have studied the binding sites of \textsuperscript{125}I ANF in homogeneous populations of human glomerular epithelial and mesangial cells (28). \textsuperscript{125}I ANF bound specifically to both cell types. Equilibrium saturation binding curves suggested one group of receptor sites in mesangial cells with a $K_{D}$ value of 99 ± 32 pM and a number of sites of 15.3 ± 3.5 fmol/mg (9,000 sites per cell). Sixteen percent of the tracer was undisplaceable in a hypotonic acid medium after 60 min of incubation at 37°C, indicating that internalization occurred for a fraction of the bound...
hormone. ANF-(1-28) and C-ANF (4-23), a specific ligand of clearance receptors, similarly inhibited [125I]ANF binding to mesangial cells, whereas [ala7-ala28]ANF, a linear analog, was slightly less potent. ANF stimulated cGMP production in glomerular epithelial cells but not in mesangial cells. [125I]ANF present in the incubation medium was degraded by mesangial cells but at a lower rate than by epithelial cells. Linear ANF-(1-28) prevented [125I]ANF from degradation, whereas thiorphan, an enkephalinase inhibitor, was inactive. We concluded from this study that human mesangial cells possess one predominant group of receptor sites that can be considered to be clearance receptors. These receptors, which participate in the catabolism of the hormone via an intracellular degradation process, are different from cell membrane-degrading enzymes, which display little activity in mesangial cells even at 37°C. The epithelial localization of the B receptors of ANF linked to guanylate cyclase in the glomerulus has been recently confirmed in vivo in the rat. Fern et al. (29) reported that after the intra-arterial administration of ANF, cGMP was demonstrated by immunofluorescence staining with a specific antibody on glomerular epithelial cells.

Mesangial cell cGMP is stimulated by nitroprusside, which acts on the soluble guanylate cyclase. This has also been demonstrated in vivo in the rat after intra-arterial administration of sodium nitroprusside. In that case, staining for immunoreactive cGMP is positive only in mesangial cells (29). A similar stimulatory effect on soluble guanylate cyclase has been demonstrated more recently for nitric oxide, identified as the endothelium-derived relaxing factor. Therefore, ANF and nitric oxide via different mechanisms produce an increase of cGMP, which counteracts the effect of vasoconstrictory agents. ANF also inhibits mesangial cell proliferation in a dose-dependent manner. The antimitogenic effect of ANF correlates with the decrement in Ca2+(30).

ECTO-5′-NUCLEOTIDASE OF MESANGIAL CELLS

5′-nucleotidase is an ectoenzyme, the catalytic site of which is located at the external surface of the plasma membrane in a variety of cells. It is a glycoprotein of 73 kd possessing at its COOH terminus a glycoprophospholipid anchor (31). This explains why 5′-nucleotidase can be released from the membrane by treatment with phospholipase C. The physiological role of 5′-nucleotidase is not entirely understood. It hydrolyzes extracellular nucleotides into the membrane-permeable nucleosides and, particularly, 5′-AMP into adenosine. The latter compound exhibits in the kidney powerful vasoactive properties. It decreases the GFR, which adapts the filtered load to the diminished capacity of tubular transport in the case of ATP depletion (32). It also produces the inhibition of renin release (33). Recently, a role for adenosine as a mediator of the tubuloglomerular feedback has been proposed (34). In addition, adenosine is a potential antiinflammatory agent that reduces the release of reactive oxygen species (35) and inhibits cell proliferation (36). For all of these reasons, it is of interest to know where 5′-nucleotidase is localized in the kidney. On rat renal sections, the enzyme is detected by histochemistry techniques in the brush border of the proximal tubular cells and in the corti-
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cal fibroblasts but not in the glomeruli (37). We have characterized 5'-nucleotidase in rat cultured mesangial cells and have studied the control of its expression. Rat mesangial cell 5'-nucleotidase is an ectoenzyme because: (1) the products of the reaction are immediately generated in the extracellular fluid, (2) identical enzyme activities are found for cultured cells in situ and sonicated cells, and (3) the diazonium salt of sulfanilic acid, which is a nonpenetrating reagent, inhibits up to 75% of the enzyme activity. The apparent $K_m$ of the nonpurified enzyme for 5'-AMP is 0.32 mM, and Mg$^{2+}$ is necessary for a full enzyme activity (38).

In a subsequent study (39), we showed that macrophage-conditioned medium produced a dose-dependent increase of mesangial cell ecto-5'-nucleotidase activity. This effect was specific for this enzyme because another ectoenzyme, Mg$^{2+}$-ATPase, remained unaffected. The effect of macrophage-conditioned medium on 5'-nucleotidase activity was apparent after 24 h and increased with time. Reversion was obtained by the withdrawal of the macrophage-conditioned medium. The stimulation of 5'-nucleotidase activity by macrophage-conditioned medium was inhibited by cycloheximide, which suggested that protein synthesis was required. Purification by gel chromatography of the factor responsible for 5'-nucleotidase stimulatory activity showed that the molecular weight of this compound was close to 20 kD. This suggested that it could be a cytokine, which was verified in studies with recombinant interleukin-1 (IL-1) and tumor necrosis factor (TNF). Both cytokines stimulated mesangial cell 5'-nucleotidase activity in a dose-dependent manner after treatment for 24 h. Maximum increases reached 4.5 and 1.7 times basal values for IL-1 (20 U/mL) and TNF-α (25 ng/mL), respectively. The effects of both cytokines were additive. Cyclooxygenase inhibitors such as indomethacin and ibuprofen inhibited approximately 50% of the effects of TNF and IL-1. Inhibition was reversed by PGE$_2$. In addition, PGE$_2$ itself produced a dose-related increase of 5'-nucleotidase activity with a maximum of 2.2 times basal value at 10 μM (40). We concluded from these results that IL-1β essentially and TNF-α to a lesser extent regulated 5'-nucleotidase expression in the plasma membrane of cultured mesangial cells and that their effects depended in part on PGE$_2$ synthesis. Because PGE$_2$ stimulates cAMP production in mesangial cells, we also studied the effect of cAMP itself and of other cAMP-stimulating agents. 8-Bromo-cAMP, an analog of cAMP able to easily cross cell membranes, stimulated mesangial cell 5'-nucleotidase activity in a dose-dependent manner (four times basal value at 500 μM). Forskolin (1 μM), PGE$_2$ (10 μM), and isoproterenol (10 μM), three products stimulating rat mesangial cell adenylate cyclase activity, enhanced cAMP accumulation within 5 min and 5'-nucleotidase activity after a lag time of 24 h. Isomethylxanthine and Ro 20-1724, two drugs inhibiting cAMP degradation, also stimulated cAMP accumulation and 5'-nucleotidase activity. As for IL-1, cycloheximide suppressed the cAMP-dependent increase of 5'-nucleotidase activity (41). Taken together, these results indicate that 5' nucleotidase expression increases in activated mesangial cells. The products of the secretion from macrophages, including IL-1, TNF, and PGE$_2$, are responsible for this change in phenotype. Such an effect is likely to result in the stimulation of local adenosine production and thus in the increased availability of adenosine at its glomerular and, more generally, cortical receptor sites.

Mesangial cells possess both the A$_1$ and A$_2$ types of adenosine receptors. Activation of A$_1$ receptors stimulates calcium entry and inhibits cAMP generation. In contrast, activation of the A$_2$ type stimulates cAMP generation and inhibits calcium entry. Exposure of mesangial cells to adenosine results in cell contraction via the A$_1$ receptors. However, adenosine, probably via cAMP generation after interaction with the A$_2$ receptors, partially blocks All-induced mesangial cell contraction (42). The effects of adenosine on mesangial cells in vitro are thus complex, and its role in glomerular physiology still needs to be clarified.

**ECTOPEPTIDASES**

Ectopeptidases are present on each glomerular cell type. Only glomerular endothelial cells exhibit All-converting enzyme activity. Glomerular visceral epithelial cells possess a variety of ectopeptidase activities. Aminopeptidase A, neutral endoproteinase, and dipeptidylpeptidase IV activities are present only in this cell line and are undetectable in mesangial cells (Table 1). In contrast, aminopeptidase N may be found at the surface of both mesangial and epithelial cells. The potential importance of membrane-bound peptidases and proteases as critical regulators of intercellular communication is now largely admitted. In particular, increased protease activity may mediate angiogenesis and tumor growth (43). Because mesangial cells play a key role in glomerular cell injury, we found it of interest to characterize aminopeptidase N in these cells (44). Aminopeptidase N is an α-aminoacylpeptidase hydrolase that splits off neutral amino acids such as alanine or leucine present at the N terminus. This enzyme has been already detected in glomeruli (45). Aminopeptidase N of human mesangial cells is an ectoenzyme as demonstrated by the extracellular location of the product, the inhibition of enzyme activity by the diazonium salt of sulfanilic acid, the similar activities measured with intact and sonicated cells, and the localization
of the immunoreactive antigen by immunofluorescence on the cell surface in parafarmaldehyde-fixed cells. This enzyme has a broad substrate specificity, which suggests that it can attack a variety of peptides. Using Ala p-nitroanilide as a substrate, we found apparent $K_m$ and $V_{max}$ values of 0.86 mM and 3.5 nmol/min/mg, respectively. Enzyme activity was maximum at pH 7.4 to 8. It was inhibited competitively by bestatin and amastatin and noncompetitively by 1,10-phenanthroline. Cell surface aminopeptidase N activity increased in the presence of phorbolmyristate acetate (PMA). This effect was time dependent and required a lag time of 12 h. It was also concentration dependent with a threshold at 1 ng/mL and a maximum stimulation (2.2 times basal value) at 10 ng/mL. The effect of PMA was prevented by cycloheximide and actinomycin. It was also suppressed by H7, an inhibitor of PKC activity. The mechanism of the effect of PMA is not a change in the cellular distribution of the enzyme but an increase in the synthesis of the enzyme units. The precise role of aminopeptidase N in glomerular mesangial cells is still unknown. Aminopeptidase N is identical to a T-cell antigen present in mice, the thymocyte-activating molecule, which has been denominated in that manner because a specific monoclonal antibody against this protein activates mouse T cells to proliferate (45). Furthermore, bestatin, an inhibitor of aminopeptidase N activity, also stimulated mouse and human T- and B-cell responses in vivo and in vitro (46). Therefore, it is possible that the mechanism by which bestatin and anti-thymocyte-activating molecule antibody stimulate immune responses or activate T cells involves the inhibition of cell surface aminopeptidase N. A number of cytokines and vasoactive peptides are synthesized in the glomeruli and behave as autacoids. Aminopeptidase N may contribute to the processing of these various peptide signals.

Even if it is not found at the surface of mesangial cells, aminopeptidase A may play a role in the control of ANF availability at its receptor sites on these cells. Aminopeptidase A, which splits off the N-terminal Asp from ANF, is present at the surface of human glomerular epithelial cells (55% of the total enzyme). Aminopeptidase A activity is calcium dependent and is inhibited by amastatin. The treatment of epithelial cells by dexamethasone increased ectoaminopeptidase A activity in a dose- and time-dependent manner. The maximal increase occurred after treatment with 0.5 μM dexamethasone for 5 days. Actinomycin D and cycloheximide prevented and RU 38486, a glucocorticoid receptor antagonist, suppressed the dexamethasone-induced increase in aminopeptidase A activity. Mineralocorticoids such as aldosterone were much less active than dexamethasone (47). Glucocorticoids can induce different enzymes such as angiotensin-converting enzyme (48) and neutral endoproteinase (49). These effects may represent mechanisms by which enzymes are regulated under physiological conditions and by which the therapeutic actions of glucocorticoids are mediated. Aminopeptidase A may modulate glomerular functions by inactivating ANF. In keeping with this possibility, Wolf et al. (50) have recently suggested that glomerular aminopeptidase A might be involved in the early regulation of the intrarenal renin-angiotensin system and might modify glomerular adaptations after renal mass ablation. Aminopeptidase A expression might be influenced by normal glucocorticoid secretion as well as by pharmacologic drug administration.

Similar to aminopeptidase A, neutral endoproteinase has been detected by immunohistochemical techniques on human renal sections only in the glomerular epithelial cells in addition to its main localization in the proximal tubular brush border (51). However, in vitro, thiorphan, a specific inhibitor of neutral endoproteinase, did not prevent ANF degra-

### TABLE 1. Main ectoenzymes of mesangial and epithelial cells

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Localization</th>
<th>Substrate</th>
<th>Products</th>
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<tbody>
<tr>
<td>5'-Nucleotidase</td>
<td>Mesangial and</td>
<td>5'-AMP</td>
<td>Adenosine and Pi</td>
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<td>epithelial cells</td>
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<tr>
<td>Aminopeptidase N</td>
<td>Mesangial and</td>
<td>Peptides with a neutral N terminal amino acid</td>
<td>Ala or Leu and a smaller peptide</td>
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<tr>
<td></td>
<td>epithelial cells</td>
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</tr>
<tr>
<td>Aminopeptidase A</td>
<td>Epithelial cells</td>
<td>Peptides with an acidic N terminal amino acid</td>
<td>Asp or Glu and a smaller peptide</td>
</tr>
<tr>
<td>Neutral endoproteinase</td>
<td>Epithelial cells</td>
<td>ANF</td>
<td>Linear ANF (cleavage at the Cys-Phe bond)</td>
</tr>
<tr>
<td>Dipeptidylpeptidase IV</td>
<td>Epithelial cells</td>
<td>Peptides with Pro or Ala at the 2nd position</td>
<td>N terminal dipeptide and a smaller</td>
</tr>
</tbody>
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