Localization of Components of the Kallikrein-Kinin System in the Kidney: Relation to Renal Function

State of the Art Lecture

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The renal kallikrein-kinin system (KKS) is a complex multienzymatic system, the main components of which are the enzyme kallikrein, the substrate kininogen, effector hormones or kinins (lysyl-bradykinin, bradykinin), metabolizing enzymes (several kininases, the most relevant being kininases I and II [ACE] and neutral endopeptidase 24.11 [NEP]), and an as-yet unknown number of activators and inhibitors of kallikrein and kininases.

The renal KKS seems to participate in both intrarenal and extrarenal complex events such as control of the extracellular volume, regulation of blood pressure, and control of sodium and water excretion, renal vascular resistance, and renin release. The biology of components of the KKS, including its regulation, molecular biology, and abnormalities associated with major diseases such as hypertension and diabetes mellitus, has been reviewed in recent years.

With regard to the main components of the KKS, it is currently known that 1) kininogens are found in large amounts in the interstitial fluid and plasma, 2) kallikrein is rapidly inactivated by several protease inhibitors when released into the circulation, 3) kinins have short half-lives in plasma, being rapidly degraded by 4) several kininases ubiquitously found in endothelial cells, the brush border of proximal convoluted tubular cells, distal tubular cells, urine, and plasma. These considerations led others to hypothesize that the KKS functions in a paracrine fashion, regulating local tissue function near where the hormones are released.

The purpose of this review is to address the cellular localization of some of the main components of the KKS in the kidney within the framework of the current morphology. Therefore, this is an anatomic approach to characterizing the sites of action and the microtopographical environments relevant to a paracrine function of the KKS.

**Microenvironments for a Paracrine Function**

Current knowledge on the morphophysiology of the kidney reveals that this is an organ of complex structure and function. The Standard Nomenclature for Structures of the Kidney released by the Renal Commission of the International Union of Physiological Sciences states that the kidney is composed of at least three different nephrons, each nephron divided into at least 10-12 different segments, any nephron segment containing multiple cell types, each cell type possibly having different and specific functions within that segment. In addition to the various tubular epithelial cells, the kidney contains cell components of the glomeruli, lymphatic system, blood vessels, and interstitium. Therefore, the kidney is composed of at least 30 different cell types, and this number may represent an oversimplification.

One might state that the requirements for paracrine function of a hormonal system in any tissue are presence of the necessary components for local generation of the hormones (enzyme and substrate), presence of receptors (or binding sites) for the hormones, the functional events related to the hormones, and presence of the metabolizing enzymes for degradation of the hormones. Based on current knowledge about the localization of components of the KKS, we postulate that there are two renal microenvironments in which the microtopographical arrangements that fulfill all the requirements for a paracrine function of the KKS exist. The microenvironments are the connecting tubule-collecting duct (Figures 1 and 2) and the juxtaglomerular apparatus (JGA) (Figure 3).

This review focuses mainly on kallikrein, kininogen, and kinins. Kininases, which degrade kinins and inactivate the KKS, are briefly discussed. Also described is the intrarenal distribution of the exogenous kallikrein inhibitor aprotinin, the administration of...
which has been widely used in attempts to inhibit activity of the KKS in a number of physiological and pathological models.

Renal Kallikrein

Current knowledge about the localization of kallikrein is the result of the use of different methodological approaches, namely, microdissected nephron segments, micropuncture, and immunolocalization.

Microdissected rabbit nephron segments reveal that kininogenase activity is restricted to the granular portions of the distal convoluted tubule and of the cortical collecting duct (together both granular portions constitute the connecting tubule in the current nomenclature). Immunoreactive kallikrein in microdissected nephron segments is concentrated mainly in the connecting tubule and to a lesser extent in the distal convoluted tubule and the cortical collecting duct. This apparently broader distribution can be explained by the transitional characteristics of the segments of the rat distal nephron, in which the connecting tubule cannot be clearly delineated and considerable overlapping of cell types occurs. Micropuncture studies in the rat also point to the distal nephron as the site of kallikrein origin and secretion.

By using ultrastructural immunocytochemistry, the presence of immunoreactive kallikrein was described exclusively in one type of cell of the rat nephron corresponding to the connecting tubule cell (CNTc). This type of cell together with the intercalated cell (Ic) comprise the components of the connecting tubule, a segment located between the distal convoluted tubule and the cortical collecting duct. (According to cytological criteria, a connecting tubule is interposed between a nephron and a cortical collecting duct. Whether this connecting tubule derives from nephrogenic blastema, and therefore must be considered a part of the nephron, or from the ureteral bud, and therefore is part of the collecting duct, remains an open question.) The CNTc is the most recently described cell type of the mammalian kidney and very little is known about its morphology and function in health or disease, yet there is increasing evidence pointing to its making a major contribution to potassium secretion in normal and pathological conditions.

So far, the only suggested functions of the CNTc are potassium secretion and the synthesis of renal kallikrein; ongoing studies are directed to elucidating how the two phenomena are related and the implications for renal function. (In the past, the renal KKS has been associated mostly with the excretion of sodium and water.)

The main ultrastructural features of the CNTc are shown schematically in Figure 1. This type of cell has a rather smooth luminal surface (in contrast with the neighboring Ic, which is rich in microfolds), a cuboid body with the nucleus localized in the midportion, and a well-developed Golgi apparatus around the lower part of the nucleus. The cytoplasm contains a number of ribosomes as polysomes and rough endoplasmic reticulum (RER) close to the Golgi apparatus. Vesicles with electrulcent contents are observed near the Golgi apparatus, near the plasma membrane, and in the cytoplasm. In the basal portion of CNTc, the plasma membrane forms prominent infoldings, most of them containing rod-shaped, elongated mitochondria. These infoldings are truly basal rather than basolateral because lateral infoldings are scarce and do not interdigitate with neighboring cells. The infoldings are in close contact with capillary blood vessels having fenestrated endothelium.

Ultrastructural immunocytochemical study reveals the presence of kallikrein in the RER, Golgi apparatus, vesicles, and plasma membranes of the CNTc. The presence of kallikrein in the RER and Golgi apparatus is consistent with synthesis of the enzyme in these cells.

Vesicles containing kallikrein are observed close to and arising from the Golgi apparatus, close to the luminal and basal plasma membranes, and in the cytosol between the Golgi apparatus and the plasmalemma. Some vesicles are in close contact or fused with the plasma membrane. With regard to the presence of kallikrein in plasma membranes, the enzyme was observed along extensive areas of the luminal and basal infoldings of the plasma membrane as reported early in purified membrane fractions. Kallikrein has been documented from the RER to the plasma membrane, and the enzyme seems to follow the classical pattern for the synthesis and secretion of glycoproteins (RER to Golgi apparatus to vesicles to membrane); however, it cannot be concluded whether the final destination of kallikrein is the membrane or secretion. Kallikrein could act in the membrane as an ectoenzyme as suggested by findings in cortical cell suspensions, and could be

![Figure 1. Schematic representation of cellular components of connecting tubule and collecting duct. Connecting tubule contains connecting tubule cell (CNTc) and intercalated cell (Ic); collecting duct contains collecting duct cell (CDc), sharing Ic with connecting tubule. Fenestrated capillary blood vessel is shown close to basal portion of the cells. Localization of kallikrein and kininogen is indicated.](http://hyper.ahajournals.org/doi/abs/10.1161/01.HYP.11-11.17?journalCode=hyp)
released into the urine and vascular compartment since it is found in both effluents of isolated perfused kidneys. Kallikrein appears to be released directly into the vascular compartment because it is present in the venous effluent of nonfiltering perfused kidneys.

It is worth noting that, in any event, kallikrein going to either the luminal or basal plasma membrane would be strategically located to act on the substrate kininogen in either the tubular fluid or the peritubular space to generate kinins, which in turn are able to influence renal function.

In the human kidney, with the same methods used in the rat nephron, kallikrein was localized in tubular segments that, according to cytological criteria, correspond to the connecting tubule. Serial sections revealed that the kallikrein-containing tubules end in the cortical collecting ducts of the medullary rays, which did not contain kallikrein, as shown in Figure 2. Close anatomic contact between the kallikrein-containing tubules and the afferent arteriole of the JGA, schematically shown in Figure 3, was consistently observed. A similar relation was demonstrated in the rat kidney by simultaneous immunocytochemical localization of renin and kallikrein in the rat renal cortex.

The close anatomic association of the kallikrein-containing cells with the renin-containing cells at the afferent arteriole close to the JGA calls for a physiological function and is consistent with a paracrine function of the KKS in the regulation of renal blood flow, glomerular filtration rate, and renin release.

In neither human nor rat normal kidneys is kallikrein observed in the proximal tubules, suggesting that kallikrein is not filtered in significant amounts at the glomerulus. This is consistent with the molecular weight and isoelectric point of the enzyme (40 kd and a pl of 4.0, respectively). Additional support for this finding is obtained from early experiments performed in isolated perfused rat kidneys in which homologous radiolabeled kallikrein did not filter through the glomerulus and in studies of clearance and metabolism of glandular kallikrein in rats in vivo.

Kallikrein, which is not filtered under normal conditions, crosses the glomerular basement membrane in pathological conditions such as chronic renal failure. In the rat remnant kidney model, kallikrein is consistently observed in reabsorption droplets of the proximal tubule and is almost certainly secondary to an alteration in glomerular permeability grossly evidenced by proteinuria.

Kininogens
Kininogens are typical secretory proteins in that their primary translation products are endowed with a transient signal sequence, and they undergo post-translational modifications (glycosylation of hydroxy and amide acceptor groups) before their export from hepatocytes and secretion into the plasma. The primary but not exclusive source of kininogen biosynthesis is the liver. Using immunofluorescence, kininogen was demonstrated in tubular cells showing a uniform location of reactivity in the cytoplasm of

Figure 2. Microtopographical arrangement of connecting tubule (CNT) and cortical collecting duct (CCD) that makes possible a paracrine function of kallikrein-kinin system in the fine regulation of sodium and water excretion.

Figure 3. Microtopographical arrangement of juxtaglomerular apparatus and connecting tubule (CNT). This is a schematic representation of observations made in humans and rats, that CNT establishes close contact with afferent arteriole (AA). Anatomic localization of kallikrein and kininase II and site of binding of kinins are indicated. CNT, proximal convoluted tubule; EA, efferent arteriole; G, glomerulus; MD, macula densa.
distal convoluted and cortical and medullary collecting ducts in human kidney,\textsuperscript{31} and immunocytochemical studies using a kinin-directed monoclonal antibody to kininogen have localized kininogens in parenchymal cells of the rat liver, in acinar and granular convoluted tubules of the submandibular gland, and in the collecting tubules of the rat kidney.\textsuperscript{32} More recently the presence of kininogen was confirmed in the collecting ducts, and it was localized in the principal cells (collecting duct cells, in the current nomenclature). Interestingly, in this study kininogen was also localized close to kallikrein in transient segments between the connecting and cortical collecting tubules of human kidney.\textsuperscript{33}

There is no information available yet on the ultrastructural localization of kininogen in the collecting duct cells, precluding the formulation of further hypotheses about its presence and intracellular processing there.

Current knowledge reveals that there are two main forms of protein secretion, namely, the regulated and the constitutive pathways.\textsuperscript{34} Proteins secreted constitutively are released in a continuous fashion commensurate with their rates of synthesis and turnover, whereas regulated proteins are packaged in a concentrated form in secretory granules and are released only on stimulation of that particular cell. Thus, the regulated secretory cell is designed to synthesize and store secretory products and to discharge rapidly a large fraction thereof in response to physiologically specific stimulation even in the absence of new protein synthesis.

The most prominent morphological characteristic of these cells is the secretory granules themselves, and it is interesting to underline that neither the CNTc (containing kallikrein) nor the collecting duct cells (containing kininogen) have classical secretory electron-dense granules (the vesicles containing kallikrein are electrolucent), suggesting (exclusively from morphological grounds) that the secretion of kallikrein and kininogen in the kidney is constitutive rather than regulated.

Newly synthesized protein traffic can be controlled, not only with respect to secretion in regulated versus constitutive secretory pathways, but also with regard to the side of a cell to which the proteins are delivered.\textsuperscript{35} Further studies are required to elucidate this issue since the implications for regulation of the KKS are of great importance in that they deal with how and where both kallikrein and kininogen exit the cells.

Kininogen has been shown to be present in renal lymph,\textsuperscript{36} and regardless of whether this kininogen originates from the bloodstream or in part also from the kidney, the interstitial generation of kinins is basically possible. Assuming that kinin generation occurs at the basolateral membrane of the CNTc or in the interstitium, one might speculate that cells further downstream (e.g., smooth muscle cells of the preglomerular and postglomerular arterioles [Figure 3]) and cortical and medullary collecting duct cells (Figure 2) would be the targets of kinins.

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**Kinin**

Activation of glandular kallikrein initiates a cascade leading to the generation of kinins, of which bradykinin is the most noted. The ability of vasodilator kinins to modulate renal function through tubular and vascular actions has been extensively investigated with the administration of bradykinin, bradykinin antagonists, antibodies against bradykinin, and inhibitors of kininases (converting enzyme inhibitors [CEI] [captopril or enalapril], inhibitors of NEP [phosphoramidon], and inhibitors of kininogenses [aprotinin]).

In the kidney, kinins have been reported to 1) increase renal blood flow and papillary blood flow,\textsuperscript{37,38} 2) mediate the hyperfiltration induced by a high-protein diet,\textsuperscript{39} 3) inhibit in isolated cortical collecting ducts the diuresis response to antidiuretic hormone\textsuperscript{40} and net sodium reabsorption\textsuperscript{41} and in inner medullary collecting duct cells conductive sodium entry,\textsuperscript{42} and 4) induce the release of renin in isolated glomeruli.\textsuperscript{43}

The kinin receptors have not been purified yet; therefore, they have not been unequivocally localized. The available studies are on bradykinin binding sites in microdissected nephron segments or membrane preparations.

In rabbits, using microdissected nephron segments, bradykinin binding was observed in the cortical collecting tubule and in the outer medullary collecting tubule; however, a small but significant binding was also observed in all other nephron segments including the glomerulus.\textsuperscript{44} In isolated perfused cortical collecting ducts, kinins had diuretic\textsuperscript{45} and natriuretic\textsuperscript{41} effects acting only on the basal side of the tubules, being inactive on the luminal side, making relevant from a physiological point of view the finding of kallikrein in the basal side of the CNTc and in isolated basolateral membranes.\textsuperscript{20,21,23}

Bradykinin also causes a dose-dependent relaxation of isolated rat afferent arterioles.\textsuperscript{45} Furthermore, a bradykinin receptor-like binding site was described in the rat glomerular membranes; the site-specificity and affinity appeared consistent with those expected of a B₂-kinin receptor.\textsuperscript{46}

The availability of competitive antagonists of B₂ receptors (K-ant)\textsuperscript{8} has provided useful tools to study the renal actions of bradykinin. Recent representative studies are discussed next. In deoxycorticosterone (DOC)-pretreated rats undergoing infusion of captopril and phosphoramidon, the administration of a K-ant causes decreases in renal plasma flow, glomerular filtration rate, and sodium excretion.\textsuperscript{47} These results point to the possibility that these effects of the K-ant may be the consequence of blockade of the renal actions of kinins that elicit renal vasodilation and increase the glomerular filtration rate and sodium excretion.\textsuperscript{47} Another approach to the problem was the use of a K-ant and Fab fragments of kinin
antibodies (BK-Fab). The advantages of BK-Fab over intact antibodies are the former's rapid distribution in the extracellular fluid where they could block kinins in the vascular-interstitial space; BK-Fab could also act in the lumen of the tubule since they are filtered and excreted into the urine.46 Using BK-Fab and a K-ant in DOC-treated rats, it was demonstrated that both decreased water excretion and K-ant decreased sodium excretion, suggesting that renal kinins acting as paracrine hormones participate in the control of water and sodium excretion.46

The more recently developed bradykinin antagonist Hoe 140 is a promising tool to address the renal effects of kinins owing to its potency (two to three orders of magnitude greater than that of previously used antagonists) and its long-lasting effect.49,50

**Kininases**

Contemporary knowledge on kininases reveals that the enzymes most active in metabolizing kinins are ACE and NEP. Both are present in the kidney, concentrated in the brush border of proximal convoluted tubules, and in urine.7,51 A major difference between them regarding their cellular localization is that ACE is present in endothelial cells whereas the amount of NEP in endothelial cells is very low.52 NEP is responsible for about two thirds of the total kininase activity in rat urine, whereas kininase I and ACE contribute only 9% and 23%, respectively.53 Therefore, NEP seems to play a major role in the catabolism of kinins, and the increase in sodium and water excretion reported after the administration of phosphoramidon could therefore be explained in part by inhibition of the intrarenal destruction of kinins.54 These results, however, should be interpreted with caution since NEP also contributes to the metabolism of other peptides, such as atrial natriuretic peptide54 and endothelin,55 with effects on sodium and water excretion, and phosphoramidon inhibits the metabolism of atrial natriuretic peptide54 and the conversion of big endothelin-1 to endothelin-1 in cultured vascular endothelial cells.56

**Aprotinin**

Aprotinin is an effective inhibitor of kallikrein (and other serine proteases) in vivo and in vitro57 and has been used to study the possible actions of the KKS under various physiological and pathological conditions.

Aprotinin significantly lowers the excretion of kallikrein, kinins, sodium, potassium, water, and prostanoids and lowers renal blood flow and the glomerular filtration rate (reviewed in Reference 43). Some of the reported effects of aprotinin are prevention of the increase in plasma renin activity induced by furosemide and anesthesia,43 reduction of the glomerular filtration rate and renal plasma flow in rats fed 50% protein,59 and reduction in the albuminuria of nephrotic rats treated with enalapril.58 Aprotinin also decreases renal plasma flow, the glomerular filtration rate, and sodium excretion in DOC-treated rats undergoing infusion of captopril and phosphoramidon,47 increases the tubuloglomerular feedback response opposing the effects induced by captopril,59 and reduces renal blood flow and increases the renal vascular resistance in rats fed a low sodium diet.60

Another study, however, has shown no effect of aprotinin at low doses (250-5,000 KIU/kg) on acute sodium and water homeostasis in anesthetized or conscious rats despite the effective inhibition of urinary kallikrein activity (amidolytic assay).61 The variable response to aprotinin could be explained by an effect of different dosages and experimental models. In fact, most of the effects of aprotinin on renal function described were investigated in either nephrotic, DOC-treated, CEI-treated rats or in rats on a low sodium or high-protein diet. Regarding the dosage, these rats received much higher priming doses (20,000-160,000 KIU/kg) and/or repeated higher doses (140,000-1,250,000 KIU/kg/day) for up to 4 days.

Despite the number of studies using aprotinin, no information is available on the intrarenal cellular distribution of exogenous aprotinin; therefore, immunocytochemical studies were conducted to elucidate this matter and to investigate whether aprotinin was acting or present at the site of kallikrein production or release.

Aprotinin localization studies were carried out with antibodies raised against pure aprotinin. The antibody recognized a single 6-kd band on immunoblots of aprotinin and of renal homogenates from aprotinin-treated rats; no staining was observed in saline-treated rats or their renal homogenates. The immunostaining for aprotinin was blocked by preincubation of the antibody with the pure antigen (unpublished observations).

Exogenous aprotinin was localized in proximal tubules, connecting tubules, and collecting tubules; no staining was observed in some proximal tubules or in glomeruli, blood vessels, or any segment of the inner medulla.62 (The presence of aprotinin in the proximal tubules, where the absorption and metabolism of polypeptides takes place, is consistent with the function of this segment.) Exogenous aprotinin colocalized with kallikrein in the CNTc of the distal nephron. This is consistent with the binding of aprotinin to kallikrein in vitro and could explain, in part, the effects of aprotinin on renal function in vivo. As mentioned before, no localization of aprotinin is found in the glomeruli that could account for its glomerular effects. Although the possibility exists that aprotinin could be acting in plasma components, pharmacokinetic studies have demonstrated that the inhibitor rapidly disappears from the blood.63

**Concluding Remarks**

We have addressed the cellular localization of some of the main components of the KKS in the kidney, within the framework of current renal morphology.
The two microenvironments proposed here, the connecting tubule–collecting duct (Figure 2) and the JGA-connecting tubule (Figure 3), are feasible on anatomic grounds, consistent with some of the described renal actions of kinins, and useful for understanding the interaction between different components of the KKS acting in a paracrine fashion. Thus, the connecting tubule and collecting duct, containing all the elements necessary for kinin generation, kinin binding sites, kininases, and functional events related to the hormones, may play a crucial role in the handling of electrolytes and water in this microenvironment. Similarly, the microtopographical arrangement composed of the JGA and connecting tubule also fulfill the requirements for a paracrine function of the KKS. The JGA-connecting tubule anatomic association, containing all the elements necessary for kinin generation, kinin binding sites, kininases, and functional events related to the hormones, may also play an important role in the regulation of renal hemodynamics and renin release.

The hypothesis of the KKS acting as a paracrine hormonal system has received further support in a different tissue with the recent findings that kallikrein messenger RNA is present in rat arteries and veins and that a vascular smooth muscle cell line cultured from rat aorta produces kallikrein, kininogen, and kininases. All the key components of the KKS are present in the arterial tissue, where they may function as an autocrine/paracrine system. Kinins liberated locally may help regulate vascular tone, and kallikrein itself, which seems to process growth factors and hormones, may help to modulate the growth and function of vascular smooth muscle cells or endothelial cells.

In summary, the components of the KKS necessary for the intrarenal production of kinins are all present within the kidney, where they can function in a paracrine fashion on glomerular and tubular events. Given the relevance of the KKS to renal function under normal and pathological conditions, it will be of great importance to obtain additional information on the mechanism of secretion and sorting of renal kallikrein and kininogen and on the purification and regulation of bradykinin receptors.

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