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Cellular Distribution of Exogenous Aprotinin in the Rat Kidney

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Aprotinin, an inhibitor of the enzymatic activity of kallikrein *in vitro*, has been used to study the possible contributions of the kallikrein-kinin systems to physiological and pathological conditions. Pharmacokinetic studies indicate that aprotinin is concentrated in the kidney; however, there is little information with regard to its cellular distribution.

The purpose of the present work was to study the cellular distribution of aprotinin, which would be valuable for a better understanding of its intrarenal effects.

Sprague-Dawley rats (200–250 g, n = 36) received aprotinin (50000 KIU/rat) and were killed at different intervals after its administration. The kidneys were examined histologically and the cellular distribution of aprotinin was studied by immunohistochemistry.

Aprotinin was localized at 30 min concentrated within vesicles in the apical border of the proximal tubule cells. Later (2 h) it was observed distributed over the cytoplasm, where it remained for the 24 h studied. Aprotinin was also detected in connecting tubule cells colocalized with kallikrein, and in the basal portion of collecting tubule cells. No evidence of endogenous aprotinin was observed. The binding of aprotinin to the connecting tubule cells and collecting ducts offers a partial explanation of its renal effects.

Key words: Collecting ducts / Connecting tubules / Immunohistochemistry / Kidney / Proximal tubules.

Introduction

Aprotinin, the broad specificity protease inhibitor of bovine origin, has been used extensively to study the contribution of the kallikrein-kinin system to various experimental physiological and pathological conditions (Carretero and Beierwaltes, 1984; Hutchison and Martin, 1990; Jaffa *et al.*, 1992; Johnston *et al.*, 1981; Kramer *et al.*, 1984; Schnermann *et al.*, 1984). Moreover, since its introduction into clinical medicine by Frey in 1953 as a treatment for acute pancreatitis (Fritz *et al.*, 1979a), it has been used in a wide variety of pathophysiological states thought to be associated with an increased protease activity (Lie *et al.*, 1989; Siebeck *et al.*, 1992). Currently, after the demonstration that the use of aprotinin significantly reduced perioperative blood loss (Davis and Whittington, 1995), high doses of the bovine polypeptide are being used in humans (Blauhut *et al.*, 1991; Havel *et al.*, 1991).

Interrelations between the kallikrein-kinin systems and other hormonal systems have been established using aprotinin. This polypeptide inhibits renin release stimulated by furosemide, and by converting enzyme inhibitors (Madeddu *et al.*, 1987; Seto *et al.*, 1983). Infusion of aprotinin significantly lowers the excretion of tissue kallikrein, kinins, sodium, potassium, water, renal blood flow and glomerular filtration rate (Seto *et al.*, 1983). Aprotinin blocks the acute antihypertensive effect of captopril in low and normal renin essential hypertensives (Overlack *et al.*, 1980), prevents the tissue kallikrein response to dietary protein (Jaffa *et al.*, 1989) and it blocks the hyperfiltration and hyperperfusion induced by aminoacid infusion (Jaffa *et al.*, 1992).

The available experimental data suggest that the renal effects of aprotinin are due to the inhibition of an enzyme that participates in the regulation of the phenomena above mentioned. Although renal kallikrein seems to be at least one of the enzymes participating in these phenomena, the non-specific nature of the inhibition and the fact that its intrarenal distribution is unclear do not allow us to draw a more definite conclusion.

Pharmacokinetic studies have demonstrated that aprotinin concentrates in the kidney (Bianchi *et al.*, 1990; Kaller *et al.*, 1978), but its exact cellular localization within this organ has not been described, although there is one report addressing the interaction of aprotinin with subcellular structures of the kidney (Just *et al.*, 1973). The lack of information on the cellular distribution of aprotinin in the kidney, contrasting with the large number of functional studies, could be due in part by the complex morphological arrangement of the renal structures (Kriz and Bankir, 1988). On the other hand, because of the cationic nature of aprotinin (pI 10.5), one may anticipate that the polypeptide could bind non-specifically to several negatively charged structures present in the kidney, namely glomerular and tubular basement membranes, and endothelial cell surfaces.

The purpose of this work was to study by immunocytochemical means the intrarenal distribution of aprotinin to obtain additional information towards understanding of its intrarenal effects.

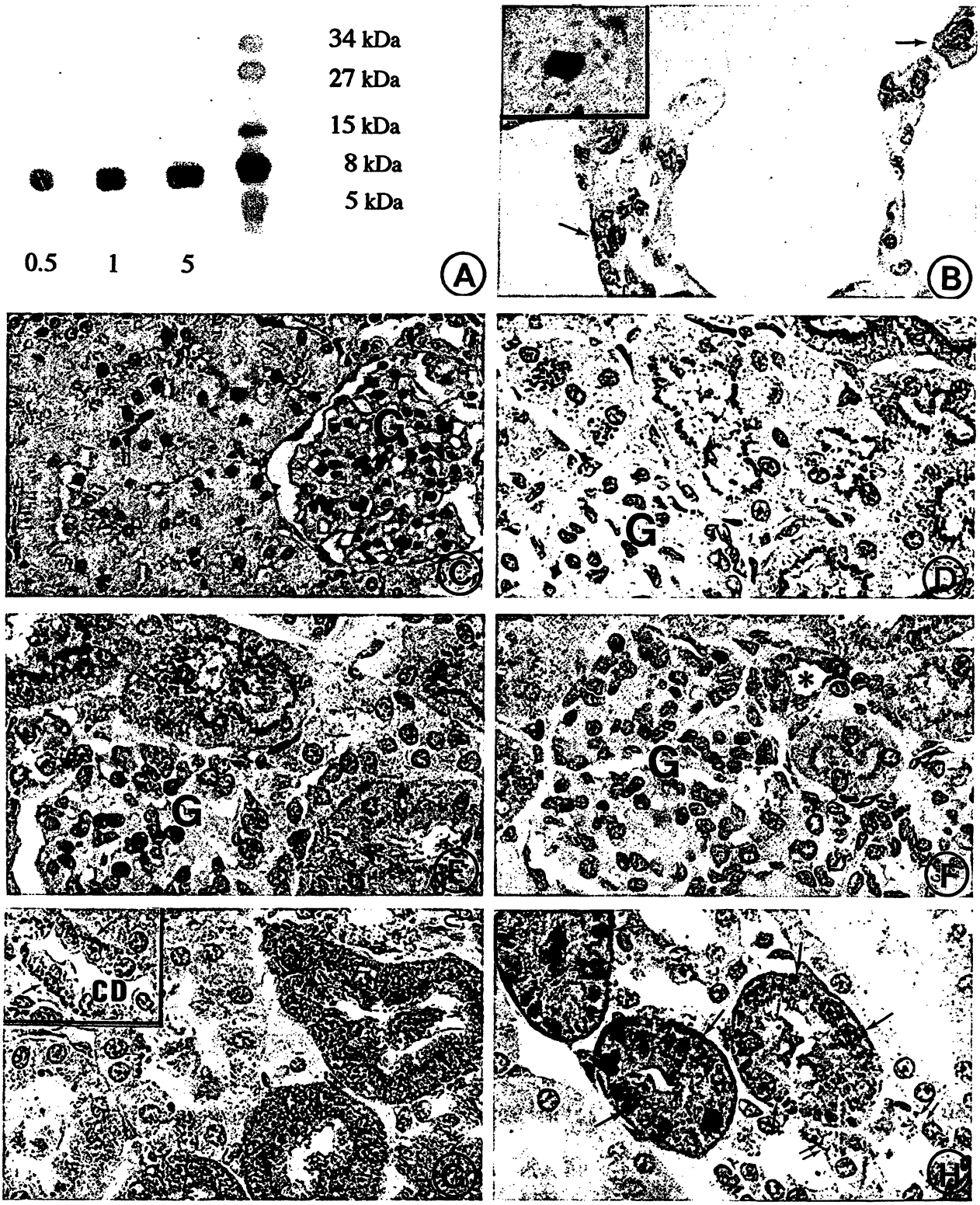

Fig. 1 Immunohistological Detection of Aprotinin in Kidney and Control Tissues.
(A) Western blot of aprotinin. Aprotinin (0.5, 1, and 5 μg) was subjected to SDS-PAGE and transferred to nitrocellulose. Antibodies against aprotinin and goat antirabbit IgG-HRP-conjugate were used to detect the polypeptide. Kaleidoscope polypeptide standards were used as molecular weight standards. (B). Localization of aprotinin in bovine lung mast cells. Bovine lung tissue was immunostained for aprotinin as a positive control for the antibodies. Note that aprotinin is present exclusively in mast cells (arrows) as confirmed by conventional toluidine blue staining (inset). (C). Control renal tissue. No staining was observed in aprotinin treated rat kidneys when the antibodies were preadsorbed with aprotinin; similarly no staining was observed in saline treated rat kidneys (not shown). (D). Renal section, 30 min after administration of aprotinin. Aprotinin is localized exclusively in endosomes at the apical portion of proximal tubule cells. Note the absence of aprotinin in glomerulus (G) and tubular basement membranes or interstitium. (E–F) Renal section, 1 h after administration of aprotinin. Aprotinin is observed in endosomes, some of which are located in the cell cytoplasm, while most of them are still in the apical pole of prox-

Results

The purity of the aprotinin preparation and specificity of the antiserum against aprotinin were checked by SDS-PAGE and Western blotting, respectively. The electrophoretic analysis by SDS-PAGE of the aprotinin preparation revealed a single band of approximately 6 kDa, and the Western blot analysis showed that the antibodies raised against aprotinin detected the 6 kDa band corresponding to aprotinin (Figure 1A).

The specificity of the antiserum is further sustained by

(i) the specific immunostaining of bovine lung mast cells, the site of origin of aprotinin, as described previously (Fritz *et al.*, 1979b; Figure 1B),

(ii) the absence of immunostaining of saline treated rat kidneys and

(iii) by abolition of the immunostaining in aprotinin treated rat kidneys when the antibodies were preadsorbed with the polypeptide (Figure 1C).

No aprotinin immunostaining was observed in kidneys from saline treated rats, whereas in aprotinin treated rat kidneys aprotinin was readily detected by the antibodies in tubular structures as a prominent staining contrasting with the unstained background. Aprotinin was concentrated in cortical structures, whereas medullary and papillary segments were devoid of the polypeptide. Within the cortex, aprotinin was located at some proximal and distal tubular segments, and was absent from glomeruli, blood vessels and perivascular or peritubular interstitium including capillaries and interstitial cells (Figures 1 D–H).

In proximal tubules, aprotinin was observed mainly in those segments located in the outer cortex and in cortical labyrinths corresponding to the more convoluted segment (segment S1) of proximal tubules. A discernible change in the subcellular distribution of aprotinin in proximal tubules was observed over the time studied after its administration. After 30 min aprotinin was localized exclusively in endocytotic vesicles in the most apical portion of proximal tubule cells (Figure 1C), at 1 h aprotinin was present also in large round-shaped endosomes shuttling to the more basal portion of the cells (Figures 1 D and E), whereas at 2 h aprotinin was distributed, with a granular aspect (endolysosomes), over the entire cytoplasm, and in lower amounts in apical endosomes of proximal tubule cells although few tubules still had aprotinin in apical endosomes (Figure 1G). After 4–8 h, aprotinin was not visible in apical endosomes of proximal tubule cells but distributed in large vesicles (endolysosomes) over the cell cytoplasm and more concentrated in the perinuclear and basal portion of the cells until at least 24 h (Figure 1H). Although aprotinin was observed consistently at 30 min and 1 h in the apical pole of proximal tubule cells, it is note-worthy that it was not seen at any time of the study in the membranes of the apical brush border but exclusively within endosomes at the base of the brush border (Figures 1D–F). Aprotinin was also observed in the basal portion of collecting duct cells located in the medullary rays of the cortex (Figure 1G). In these collecting ducts, aprotinin was observed only in the basal portion of the cells and not in the apical pole becoming visible 2 h after its administration and remaining there for at least 24 h although the intensity decreased with time, and also few vesicles were observed over the cytoplasm (Figure 1H).

As mentioned before, aprotinin displayed a regional distribution in the kidney, being localized exclusively in the cortex and cortical labyrinths (Figure 2 A–D). Within the cortex, in contrast to the staining pattern observed in proximal tubules (i.e. endosomes and lysosomes), aprotinin was observed at 30 min and 1 h after its administration at apical plasma cell membranes, and also in the cell cytoplasm of scattered tubules (Figure 1F) which, according to morphology and kallikrein colocalization (Figure 2 E and F), corresponded to connecting tubules, the site of kallikrein origin.

Discussion

From a morphofunctional point of view, three aspects related to the distribution of aprotinin in the kidney have to be discussed. First, the localization of the polypeptide in specific structures of the kidney, second, the metabolic pathway of aprotinin in the kidney and third, the anatomic basis for its physiological actions in the kidney.

Because of the physicochemical characteristics of aprotinin (i.e. low molecular weight and basic isoelectric point) and its carbohydrate-binding property (Davis *et al.*, 1976) we expected *a priori* to find aprotinin associated with renal structures bearing negatively charged surfaces or free uranyl and sialosyl groups like the glomeruli, blood vessels, basement membranes and many cortical and medullary segments. However, aprotinin showed a very selective distribution restricted to proximal convoluted tubules and to distal tubules and collecting ducts. These results suggest that additional factors should be considered. Indeed, other polypeptides with an isoelectric point similar to that of aprotinin such as bovine cytochrome c (pI 10.6, 12.4 kDa) do not accumulate in the rat kidney and are rapidly cleared from this tissue, whereas lysozyme, an anionic peptide (pI of 11, 14.3 kDa), accumulates in the kidney (Bianchi *et al.*, 1990).

imal tubule cells. Note the aprotinin staining in the luminal membrane and cytoplasm of a distal/connecting tubule (asterisk) close to a glomerulus (G). (G) Renal section, 2 h after administration of aprotinin. Most of the aprotinin immunostaining is localized in vesicles (endolysosomes) over the cytoplasm of proximal tubule cells. Note the presence of aprotinin in the basal portion of collecting duct cells (CD, inset). (H) Renal section 24 h after the administration of aprotinin. Most of the aprotinin immunostaining is present in endolysosomes located in the perinuclear and basal portion of proximal tubule cells (single arrows). In the collecting duct cells, aprotinin is present in small vesicles mostly concentrated at the basal portion of the cells (double arrows), although some vesicles seem to be in the cytoplasm.

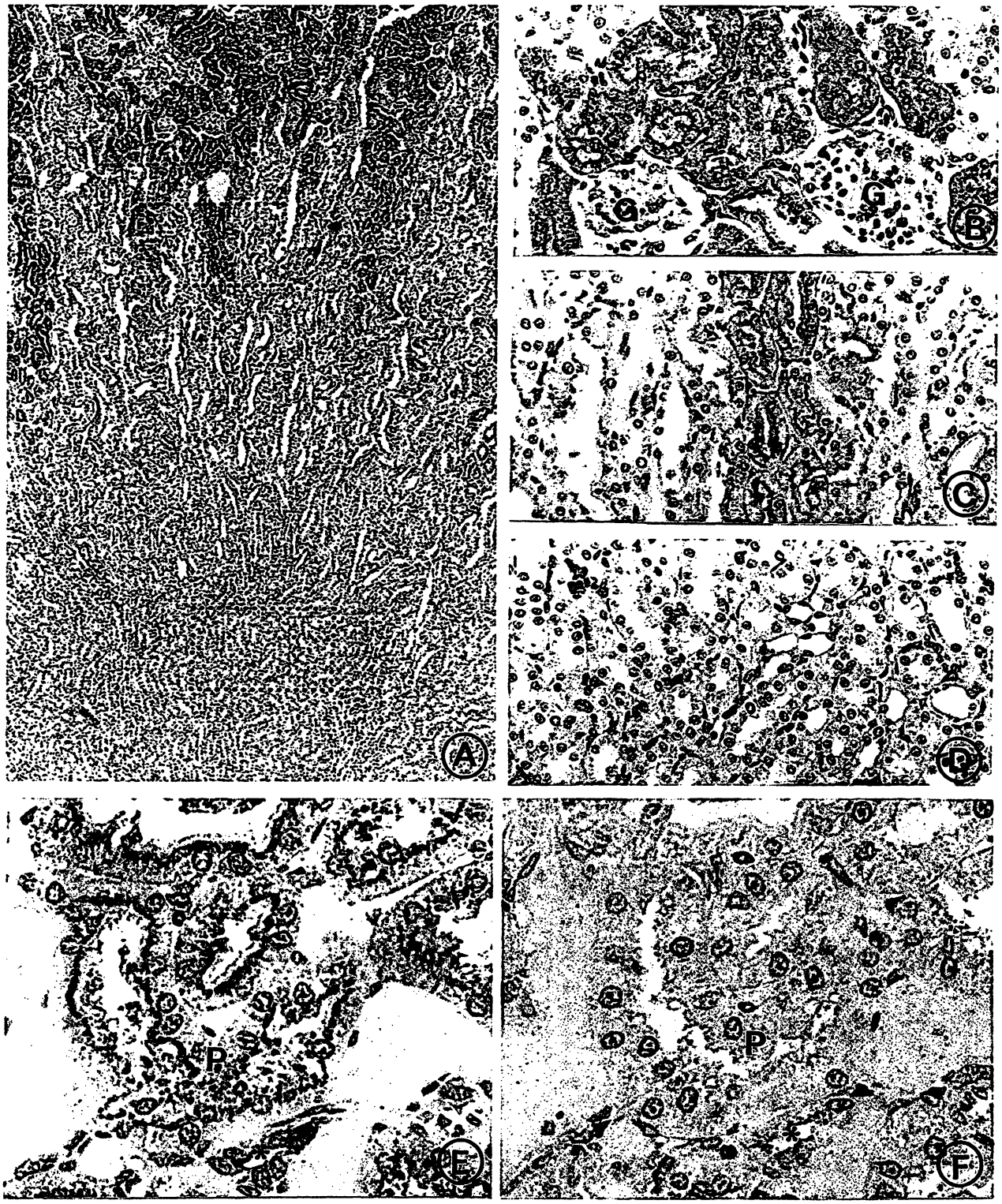

Fig. 2 Regional Distribution of Aprotinin in the Kidney.
Low power magnification of a kidney section including cortex and medulla, immunostained for aprotinin 1 h after its administration. Note that aprotinin localization is restricted to the cortex and cortical labyrinth, white it is being absent in the medulla. The dotted squares indicate the zones of the kidney enlarged in the Figures on the right; the upper square corresponds to the cortex (B), the middle square corresponds to the cortical labyrinth (C), and the lower square corresponds to the medulla (D). Note that aprotinin is observed only in the cortex and cortical labyrinth (2A, B and C), and is absent from the medulla (2A and D).
(E) and (F) Immunohistochemical localization of aprotinin (E) and kallikrein (F) in adjacent serial sections. In (E), aprotinin is present in proximal tubules (P) and in a connecting tubule (asterisk), in the adjacent section (F) kallikrein is present in the same connecting tubule (asterisk) indicating colocalization of aprotinin and kallikrein in the connecting tubule cells. No kallikrein is present in proximal tubules (P).

Aprotinin was not observed in vehicle treated rat kidneys excluding the presence of an endogenous polypeptide structurally similar to the bovine inhibitor. A previous report described the existence of an endogenous kallikrein-specific inhibitor in rat kidney tubules, (Geiger and Mann, 1976), obviously its nature is different from the bovine polypeptide

The main site of renal accumulation of aprotinin was the proximal tubule, precisely its more convoluted segment (S1). The dynamics of aprotinin distribution in the proximal tubules showed its presence as early as 30 min after administration in endocytotic vesicles or endosomes at the luminal pole of the cells, in round-shaped endosomes shuttling to the more basal portion of the cells after 60 min, and distributed over the cytoplasm of proximal tubule cells after 4 h. This cellular and subcellular distribution pattern of aprotinin is consistent with the described function of this segment, i.e. absorption and metabolization of polypeptides (Bianchi *et al*., 1990; Moestrup *et al*., 1995). Polypeptides can be metabolized in the proximal tubule either by luminal hydrolysis by brush border proteases followed by reabsorption of the resulting amino acids, or by luminal reabsorption via endocytosis followed by lysosomal degradation (Maack *et al*., 1992). Our results show that aprotinin follows the endocytic pathway since it was observed within intracellular vesicles and was absent from the brush border membranes. The lack of hydrolysis by brush border enzymes is not surprising considering the presence of disulphide bonds in aprotinin. In fact, peptide hormones such as insulin (6 kDa, two chains with disulphide bonds) and smaller peptides possessing disulphide bonds such as oxytocin resist luminal hydrolysis and are reabsorbed via endocytosis followed by lysosomal degradation (Maude *et al*., 1981, Peterson *et al*., 1977) whereas linear polypeptides without disulphide bond such as glucagon (5.6 kDa) undergo luminal hydrolysis in the proximal tubule (Peterson *et al*., 1982).

The presence of intracellular aprotinin for at least 24 h suggests a slow tubular metabolism, which is in agreement with electrophoretic studies showing a single 6 kDa aprotinin-immunoreactive band in kidney homogenates detectable for at least 24 h (Oestreicher, Olavarria, Vio, unpublished), indicating that the administered aprotinin remained intact.

In addition to proximal tubules, aprotinin was also observed in the basal (peritubular) portion of collecting ducts. This localization should be also related to polypeptide metabolism since peritubular metabolization of polypeptides such as insulin and parathyroid hormone has been previously described (Martin *et al*., 1977; Peterson *et al*., 1982).

In regard to the anatomic basis of the physiological actions of aprotinin in the kidney, the fact is interesting that the polypeptide was localized in the connecting tubule cells of the distal nephron from 60 min onwards to 4 h after its administration. The presence of aprotinin at the site of kallikrein synthesis could explain in part the effects of aprotinin on renal function *in vivo*. Aprotinin may inhibit kallikrein at its site of synthesis or release, thus interfering with renal function. From an anatomic point of view and based on the localization of the components of the kallikrein system, we proposed previously the existence of two renal microenvironments for a contribution of the kallikrein system to the regulation of renal function (Vio *et al*., 1992), one composed of the connecting tubule and collecting duct (CNT-CCD) and the other by the juxtaglomerular apparatus and connecting tubule (JGA-CNT). The former participates in the resorption of electrolytes and water, whereas the later participates in the regulation of renal hemodynamics and renin release (Vio *et al*., 1992). The binding of aprotinin to the connecting tubule could account for most of the described effects of aprotinin on renal electrolyte excretion (Kramer *et al*., 1984, Seto *et al*., 1983), on the renal prostaglandin system (Nasjletti *et al*., 1978), on renal hemodynamics and renin release (Carretero and Beierwaltes, 1984; Jaffa *et al*., 1992), and on tubuloglomerular feedback response (Schnermann *et al*., 1984).

Among the effects of aprotinin not related to the kallikrein system, reports that the administration of aprotinin reduces the urinary excretion of epidermal growth factor (EGF) while the amount of renal EGF increases indicate that the renal precursor of EGF, which is located in the distal tubule, is processed by a proteinase that can be inhibited by aprotinin (Jorgensen *et al*., 1990). Therefore, in the distal tubules aprotinin could have a local inhibitory effect on the renal processing of EGF.

In addition to kallikrein, aprotinin could be bound to esterase A, a closely related kinin-generating serine protease inhibited by aprotinin. However, esterase A is present primarily in the basolateral region of both proximal and distal straight tubules of the outer medulla and medullary rays (Simson *et al*., 1988) and aprotinin was not observed at these sites.

In summary, aprotinin binds selectively to early proximal tubules, connecting tubule cells where kallikrein is synthesized, and the basal portion of some collecting tubules. The presence of aprotinin in proximal convoluted tubules is consistent with the role of the kidney as the main place for polypeptide metabolism, whereas its localization in distal tubular segments (where kallikrein is synthesized and secreted) offers an explanation for some functional renal effects. The meaning of the presence of aprotinin in the basal portion of collecting ducts is unknown and could also be related to inhibition of kallikrein, as this enzyme is found in the tubular fluid and peritubular space.

Materials and Methods

Materials

Aprotinin (Trasylol) was purchased from Bayer AG, (Wuppertal, Germany); goat anti-rabbit IgG and PAP complex were purchased from Organon-Teknica, Cappel, (West Chester, PA, USA), and Sternberger-Meyer Immunochemicals Inc., (Jarrestville, MD, USA). Freund's adjuvant, carrageenan, and Triton X-100 were

from Sigma (St. Louis, MO, USA), and Tris, phosphates and all other chemicals from Merck (Darmstadt, Germany).

Animals and Tissue Processing

The animals were maintained at the university animal care facilities and the experimental procedures were in accordance with institutional and international guidelines for the welfare of animals. Male Sprague-Dawley rats (200–250 g, b.wt., n = 36) received a single dose of aprotinin (50 000 KIU/rat) and were sacrificed under anaesthesia (pentobarbital 60 mg/kg) at 30 min and 1, 2, 4, 8, 24 h after the single injection. Control rats received an equivalent volume of sterile saline (n = 12). The dose of aprotinin used in our study (50 000 KIU/rat) was selected from the dosages used by other investigators ranging from 50 000 KIU/rat/day to 250 000 KIU/rat (Nasjletti *et al.*, 1978; Hutchison and Martin, 1990). Fifty ml vials containing sterile aprotinin (Trasylol, 500 000 KIU/vial) were concentrated by ultrafiltration under nitrogen by an Amicon stirring cell with a YM1 membrane (1 kDa cut-off) to yield a concentration of 50 000 KIU/0.25 ml.

The kidneys were removed for morphological analysis. The tissue was cut following the cortico-papillary axis in 2 mm thick pieces, including cortex and medulla, fixed by immersion in Bouin's fluid for 24 h, dehydrated, embedded in Paraplast plus (Monoject Scientific, St. Louis, USA), sectioned at 7 μm thickness in a microtome, mounted on glass slides and stored.

Immunohistochemistry

The immunostaining procedure used was the unlabeled antibody method (Sternberger, 1986), as previously modified to determine the cellular localization of tissue kallikrein in the rat and human kidneys (Vio and Figueroa, 1985; Vio *et al.*, 1988). The paraplast was removed with xylene and the tissue was rehydrated with a series of alcohol solutions. The tissue sections were rinsed in 0.05 M Tris-phosphate saline (TPS) pH 7.6, and were incubated with rabbit antiserum against aprotinin (1:10 000) for 18 to 24 h; goat antiserum against rabbit IgG (1:20) for 30 min; PAP complex of rabbit origin (1:150) for 30 min followed staining with 3-3'-diaminobenzidine (0.01%) plus hydrogen peroxide (0.03%). The colocalization of aprotinin and kallikrein was examined in serial consecutive sections by immunostaining of alternate sections with antibodies against aprotinin (1:10 000) and rat urinary kallikrein (1:5 000). The antisera and PAP complex were diluted in TPS containing 0.5% Triton X-100 and 0.7% lambda-carrageenan. The incubations were done in glass staining jars at 22 °C and between each incubation the tissues were rinsed with TPS (Velarde *et al.*, 1995). The tissue sections were observed and photographed on a Nikon Optiphot microscope with a Nikon Microflex UFX IIA photographic system (Nikon Corporation, Tokyo, Japan).

Antisera against aprotinin were obtained by immunization in rabbits by intradermal injections of aprotinin (200 μg/animal) emulsified in complete Freund's adjuvant, followed by booster injections every 3–4 weeks. Blood was obtained prior to the immunizations (preimmune serum), and during the immunization protocol in periodical bleeding to test the antibody titer. The purity of aprotinin and the specificity of the antibodies were studied by electrophoresis and Western blot analysis. Aprotinin (0.5, 1, and 5 μg) was electrophoresed in sodium dodecyl sulphate-polyacrylamide gels (18% SDS-PAGE) in a Mini-Protean II cell (Bio-Rad Laboratories, Richmond, California USA) and transferred onto 0.2 μm nitrocellulose membranes. The nitrocellulose membranes were blocked with 1% BSA and incubated overnight with antibodies against aprotinin at a 1:5 000 dilutio. The protein antibody complex was detected with goat anti-rabbit IgG HRP conjugate and developed with 4-chloronaphtol and H_2O_2 (Towbin *et al.*, 1979). Kaleidoscope polypeptide standards (Bio-Rad) were used as molecular weight standards.

Immunohistochemical controls: preadsorption of the antiserum with aprotinin (1.4 μg aprotinin/μl of antiserum) and replacement of the primary antiserum with preimmune serum were used as immunocytochemical controls. As a positive control for aprotinin, bovine lung tissue (obtained from a slaughterhouse) was used processed similarly to the rat tissue.

Acknowledgements

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References

Bianchi, C., Donadio, C., Tramonti, G., Auner, I., Lorusso, P., Deleide, G., Lunghi, F., Vannucci C., Vitali. S., Ricchiuti, V., and Guzzardi, R. (1990). High and preferential accumulation in the kidney of anionic and cationic small proteins. Contr. Nephrol. *83*, 39–46.

Blauhut, B., Gross, C., Necek, S., Doran, E., Spath, P., and Lundsgaard-Hanseb, P. (1991). Effects of high-dose aprotinin on blood loss, platelet function, fibrinolysis, complement, and renal function after cardiopulmonary bypass. J. Thorac. Cardiovasc. Surg. *101*, 985–967.

Carretero, O.A., and Beierwaltes, W.H. (1984). Effect of glandular kallikrein, kinins and aprotinin (a serine protease inhibitor) on renin release. J. Hypertens. *2*, 125–130.

Davis, H., Gascho, C., and Kiernan, J.A., (1976). Effects of aprotinin on organ cultures of rat kidney. In Vitro *12*, 192–197.

Davis, R., and Whittington, R. (1995). Aprotinin. A review of its pharmacology and therapeutic efficacy in reducing blood loss associated with cardiac surgery. Drugs *49*, 954–983.

Fritz, H., Fink, E., and Truscheit, E. (1979a). Kallikrein Inhibitors. Federation Proc. *38*, 2753–2759.

Fritz, H., Kruck, J., Rüsse, I., and Liebech, G. (1979b). Immunofluorescence studies indicate that the basic trypsin-kallikrein-inhibitor of bovine organs (Trasylol) originates from mast cells. Hoppe-Seyler's Z. Physiol. Chem. *360*, 437–444.

Geiger, R., and Mann, K. (1976). A kallikrein specific inhibitor in rat kidney tubules. Hoppe-Seyler's Physiol. Chem. *357*, 553–558.

Havel, M., Teufelsbauer, H., Knobl, P., Dalmatiner, R., Jaksch, P., Zwolfer, W., Muller, M., and Vukovich, T. (1991). Effect of intraoperative aprotinin administration on postoperative bleeding in patients undergoing cardiopulmonary bypass operation. J. Thorac. Cardiovasc. Surg. *101*, 968–972.

Hutchison, F.N., and Martin, V.I. (1990). Effects of modulation of renal kallikrein-kinin system in the nephrotic syndrome. Am. J. Physiol. *258*, F1237–F1244.

Jaffa, A.A., Harvey, J.N., Sutherland, S.E., Margolius, H.S., and Mayfield, R.K. (1989). Renal kallikrein responses to dietary protein: A possible mediator of hyperfiltration. Kidney Int. *36*, 1003–1010.

Jaffa, A.A., Vio, C.P., Silva, R.H., Vavrek,.R.J., Stewart, J.M., Rust, P.F., and Mayfield, R.K. (1992). Evidence for renal kinins as mediators of amino acid-induced hyperperfusion and hyperfiltration in the rat. J. Clin. Invest. *89*, 1460–1468.

Johnston, P.A., Perrin, N.S., Bernard, D.B., and Levinsky, N.G. (1981). Control of rat renal vascular resistance at reduced perfusion pressure. Circ. Res. *48*, 734–739.

Jorgensen, P.E., Raaberg, L., Poulsen, S.S., and Nexo, E. (1990). The urinary excretion of epidermal growth factor in the rat is reduced by aprotinin, a proteinase inhibitor. Reg. Peptides *31*, 115–124.

Just, M., Torok, P., and Habermann, E. (1973). Interaction of Trasylol with subcellular structures of the kidney. In: Kininogenases, Kallikrein., G.L. Haberland and J.W. Rohen, eds. (Stuttgart, Germany: F.K. Schattauer Verlag), pp. 123–127.

Kaller, H., Patzschke, K., and Wegner, L.A. (1978). Pharmacokinetic observations following intravenous administration of radioactive labelled aprotinin in volunteers. Eur. J. Drug Metab. *2*, 79–85.

Kramer, H.J., Düsing, R., Glänzer, K., Kipnowski, J., Klingmüller, D., and Meyer-Lehnert, H. (1984). Effects of aprotinin on renal function. Contr. Nephrol. *42*, 233–241.

Kriz, W., and Bankir, L. (1988). A standard nomenclature for structures of the kidney. Kidney Int. *33*, 1–7.

Lie, T.S., Seger, R., Hong, G.S., Preissinger, H., and Ogawa, K. (1989). Protective effect of aprotinin on ischemic hepathocellular damage. Transplantation *48*, 396–399.

Maack, T., Park, C., and Camargo, M.J. (1992). Renal filtration, transport, and metabolism of proteins. In: The Kidney: Physiology and Pathophysiology, D.W. Seldin and G. Giebish, eds. (New York, USA: Raven Press), pp. 3005–3038.

Madeddu, P., Oppes, M., Soro, A., Dessi-Fulgheri, P., Glorioso, N., Bandiera, F., Manunta, P., Rubattu, S., and Rappelli, A. (1987). The effects of aprotinin on renin release and urinary sodium excretion in mild essential hypertensives. Hypertension *5*, 581–586.

Martin, K., Hruska, K.A., Lewis, J., Anderson, C., and Slatopolski, E. (1977). The renal handling of parathyroid hormone in the dog: The role of peritubular uptake and glomerular filtration. J. Clin. Invest. *60*, 808–814.

Maude, D.L., Handelsman, D.G., Babu, M., and Gordon, E.E. (1981). Handling of insulin by the isolated perfused rat kidney. Am. J. Physiol. *240*, F288–F294.

Moestrup, S.K., Cui, S., Vorum, H., Bregengard, C., Bjorn, S.E., Norris, K., Gliemann, J., and Christensen, E.I. (1995). Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. J. Clin Invest. *96*, 1404–1413.

Nasjletti, A., McGiff, J.C., and Colina-Chourio, J. (1978). Interrelation of the renal kallikrein kinin system and renal prostaglandins in the conscious rat. Circ. Res. *43*, 799–806.

Overlack, A., Stumpe, K.O., Heck, I., Ressel, C., Künhert, M., Krück, F. (1980) Identification of angiotensin II- and kinin-dependent mechanisms in essential hypertension. In: Hypertension: Mechanisms and Management. T. Philip and A. Distler, eds. (Berlin, Germany: Springer Verlag), pp. 183–190.

Peterson, D.R., Carone, F.A., Oparil, S., and Christensen, E.I. (1982). Differences between renal tubular processing of glucagon and insulin. Am. J. Physiol. *242*, F112–F118.

Peterson, D.R., Oparil, S., Flouret, G., and Carone, F. (1977). Handling of angiotensin II and oxytocin by renal tubular segments perfused in vitro. Am. J. Physiol. *232*, F319–F324.

Peterson, J., Kitaji, J., Duckworth, W.C., and Rabkin. R. (1982). Fate of ^{125}I insulin removed from peritubular circulation of isolated perfused kidneys. Am. J. Physiol. *243*, F126–F132.

Schnermann, J., Briggs, J.P., Schubert, G., and Marin-Grez, M. (1984). Opposing effects of captopril and aprotinin on tubuloglomerular feedback response. Am. J. Physiol. *247*, F912–F918.

Seto, S., Kher, V., Scicli, A.G., Beierwaltes, W., and Carretero, O.A. (1983). The effect of aprotinin (a serine protease inhibitor) on renal function and renin release. Hypertension *5*, 893–897.

Siebeck, M., Spannagl, M., Hoffmann, H., and Fink, E. (1992). Effects of protease inhibitors in experimental septic shock. Agents Actions. *38* (suppl III), 421–427.

Simson, J.A., Condon, J.L., Chao, L., Chao, J. (1988). Comparison of the distribution of tissue kallikrein and esterase A, a kallikrein-like enzyme, in rat kidney using specific monoclonal antibodies. J. Histochem. Cytochem. *36*, 1251–1254.

Sternberger, L.A.,(1986). The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In: Immunocytochemistry, L.A. Sternberger, ed. (New York: John Wiley), pp. 90–209.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheers: procedure and some applications. Proc. Natl. Acad. Sci. USA. *76*, 4350–4354.

Velarde, V., Humphreys, J., Figueroa, C.D., and Vio, C.P. (1995). Postnatal maturation of kallikrein-producing cells (connecting tubule cells) in the rat kidney. A morphometric and immunohistochemical study. Anat. Embryol. *192*, 399–406.

Vio, C.P., and Figueroa, C.D. (1985). Subcellular localization of renal kallikrein by ultrastructural immunocytochemistry. Kidney Int. *28*, 36–42.

Vio, C.P., Figueroa, C.D., and Caorsi, I. (1988). Anatomical relationship between kallikrein-containing tubules and the juxtaglomerular apparatus in the human kidney. Am. J. Hypertens. *1*, 269–271.

Vio, C.P., Loyola, S., and Velarde, V. (1992). Localization of components of the kallikrein-kinin system in the kidney: Relation to renal function. Hypertension. *19*, 10–16.

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