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Summary. By means of immunohistochemical reactions, the authors proved the inhibitor II-related immunoreactivity in distal convoluted tubules of human kidney. A sharp inhibitor II-related immunoreactivity was also present in the blood vessels' wall. On the contrary, in the wall of proximal tubules and glomeruli only low reactivity was found. The demonstration of an inhibitor II-related immunoreactivity in the distal convoluted tubules and vessels of human kidney represents a strong evidence that an inhibitor of kallikrein exists and acts also in humans as an important key in the kallikrein-renin-angiotensin-aldosterone chain and hitherto confirms the experimental data of the literature. The proved inhibitor in the human kidney may intervene in the modulation of the kallikrein-kinin system and thus represents a key role in the intrarenal mechanisms related to the blood flow and arterial pressure regulation.

Key words. Aldosterone, angiotensin, arterial hypertension, kallikrein, kinine, inhibitor, kidney.

Riassunto. *Importanza di un inibitore della callicreina nel rene umano. Un nuovo anello della catena callicreina-renina-angiotensina-aldosterone.*

Gli autori descrivono i risultati di uno studio immunoistochimico che ha messo in evidenza un'immunoreattività correlata all'inibitore-II localizzata in corrispondenza dei tubuli convoluti distali del rene umano ed una debole immunoreattività anche nei vasi ematici. Al contrario, solo una scarsa immunoreattività è stata rilevata nella parete dei tubuli prossimali e dei glomeruli. L'immunoreattività positiva per l'inibitore-II nei tubuli convoluti distali e nei vasi ematici dimostra l'esistenza, anche nell'uomo, di un inibitore della callicreina quale importante elemento della catena callicreina-renina-angiotensina-aldosterone. Questo fattore inibitore potrebbe intervenire nel processo di modulazione del sistema callicreina-chinina e quindi nel meccanismo di regolazione del flusso ematico renale e della pressione arteriosa.

Parole chiave. Aldosterone, angiotensina, callicreina, chinina, inibitore, ipertensione arteriosa, rene.

Introduction

The renin-angiotensin, prostaglandin and kallikrein-kinin systems show interrelated actions and participate in the control of blood pressure by regulating intrarenal sodium, potassium, and water excretion, by modifing the tone of extrarenal blood vessels and perhaps by regulating renal blood flow distribution.

The renin-angiotensin system consists of two main enzymes, renin and angiotensin converting enzyme (ACE). Renin acts on angiotensinogen to form angiotensin I and ACE acts on angiotensin I to form angiotensin II. Angiotensin II stimulates the synthesis of aldosterone and mediates vasoconstriction and sodium retention [2,13,14,30]. The third step is the formation of angiotensin III, a heptapeptide, from angiotensin II [20,22]. Angiotensin II and III are two potent pressor and aldosteronogenic peptides.

They act on arteriolar smooth muscle cells causing vasoconstriction and participate in the regulation of blood pressure. On the other hand, angiotensin has a critical role in many forms of hypertension and represents the major stimulator of aldosterone secretion [3].

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The renal kallikrein-kinin system is the least well defined of these systems. Kallikreins are serine proteases that release kinins from kininogens, which stimulate the synthesis of prostaglandins and mediate vasodilatation and natriuresis [3,34]. Over 90% of kidney kallikrein is found in the renal cortex while just a small amount is found in the medulla [25]. Moreover, there is a different distribution of kallikrein in the various portions of the renal cortex. The amount of kallikrein gradually decreases from the outer to the inner cortex, glomeruli contain a small amount of kallikrein in relation to the total cortex [5,21].

Immunohistochemically, it has been demonstrated in rats and humans that kidney kallikrein is localized in the convoluted distal tubules [21] and it is likely that kallikrein is synthetized in the distal nephron [5,28]. In the proximal tubules of the rat and human kidney, kallikrein was observed as reabsorption droplets [10]. Vanhoutte et al. [33] demonstrated the existence of a local kallikrein-kinin system in the vascular wall, too. Urinary kinins may be formed in the distal part of the nephron [26,27].

Kallikreins release kinins from kininogens. Kinins are peptides with multiple and different pharmacological actions. It has been reported that both urinary kallikrein and kinins can stimulate release of renin [29] and that aprotinin, a serine protease inhibitor, is able to block the effect of urinary kallikrein on renin release [18]. Kallikrein release may be stimulated by prostaglandins [19] and also by angiotensin [16]. The kallikrein-kinin, renin-angiotensin-aldosterone and prostaglandins systems are linked to each other and further connecting rings not yet known are likely to exist.

There are considerable amounts of protease inhibitors in plasma that could rapidly inactivate serine proteases such as tissue kallikrein. A kallikrein inhibitor has been demonstrated in the tubules of the rat kidney [11]. This inhibitor may have a role in the mechanism of formation of kinins in the kidney. A group of serine protease inhibitors, structurally and functionally similar to bovine pancreatic trypsin inhibitor (BPTI or aprotinin) has been demonstrated in bovine organs [6].

BPTI (inhibitor IV or Kunitz inhibitor) and three other protease inhibitors (I, II and III), which inhibit the kallikrein and other proteolytic enzymes (trypsin and chymotrypsin) have been demonstrated in bovine spleen. Inhibitor II, in particular, has a protein structure constituted by 58 amino acids as many as BPTI [9], but with seven substitutions along the sequence. Some of these substituted amino acids are in a key position for the inhibition of kallikrein.

These inhibitors were till now not demonstrated in humans. It may be, of course, presumed that a protease inhibitor II of the bovine exists also in humans. This inhibitor may play an important physiological role in the kallikrein-renin, renin-angiotensin and prostaglandins systems. We decided therefore to study the existence of such a inhibitor of kallikrein in the tubules of human kidney.

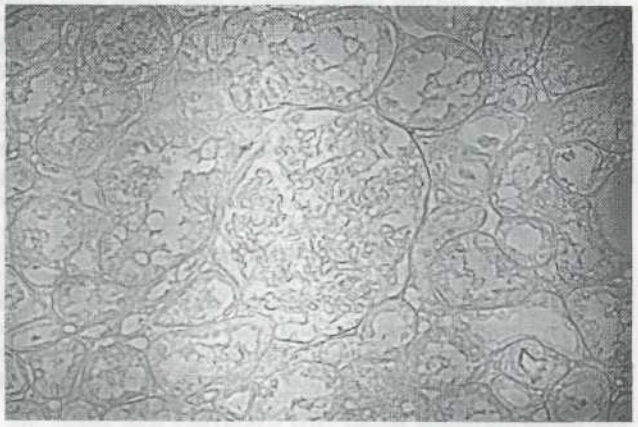

Figura 1A. Control performed by substituting specific immunoglobulins with PBS-BSA.

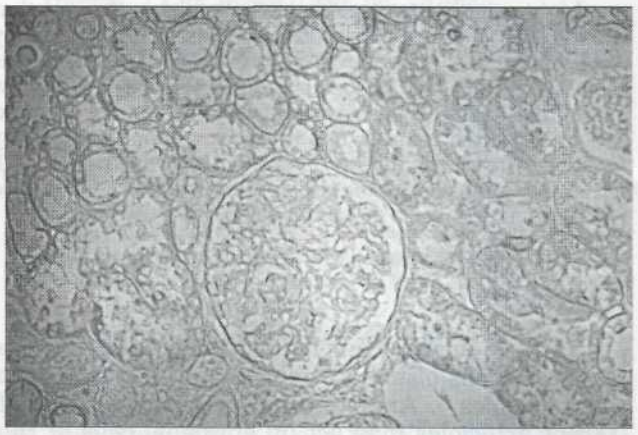

Figura 1B. Antibodies against inhibitor II of kallikrein in human kidney. Inibitor II - related immunoreactivity predominantly located to distal but also to proximal convoluted tubules.

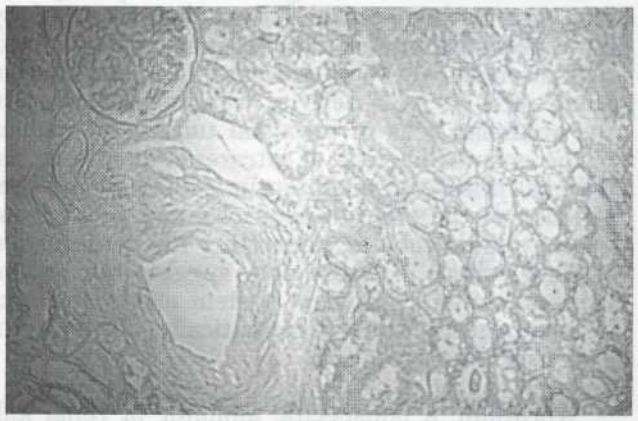

Figura 1C. Antibodies against inhibitor II of kallikrein in human kidney. Immunoreactivity is also present in the wall of blood vessels.

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For the immunohistochemical experiments, small pieces of human kidney were taken from three patients who underwent nephrectomy for renal cell carcinoma. Specimens of normal tissue (distant more than 5 cm from the external border of the tumour) were fixed by immersion in Bouin fluid for 24-36 hours, dehydrated through an ethanol series and embedded in paraffin. Sections (4-5 μm thickness) were mounted on glass slides and incubated with the desired specific immunoglobulins, which recognized inhibitor II, obtained by one of us, diluted in PBS-BSA. Section-bound antibodies were detected using biotinylated second antibody and streptavidin-biotinylated peroxidase complex followed by treatment with 3,3,-diamino-benzidene and hydrogen peroxidase [18]. In order to exclude any background staining due to the presence of endogenous peroxidase, sections were pretreated with 0.5% hydrogen peroxidase and methanol for 30 min, and 0.2% hydrochloric acid and ethanol for 10 min. Controls were performed by substituting specific immunoglobulin preparations with PBS-BSA.

Results

Light microscope immunohistochemistry showed that about 90% of distal convoluted tubules of human kidney contain inhibitor II-related immunoreactivity, which is selectively localized to parietal level (figure 1A). A low inhibitor-II-related immunoreactivity in the wall of proximal convoluted tubules, and a very low immunoreactivity was demonstrated at the level of glomeruli (figure 1B). A sharp inhibitor-II-related immunoreactivity was also present in the wall of blood vessels (figure 1C).

Discussion

In rats, angiotensin II and renin coexist in the epithelial cells of the afferent and efferent glomerular arterioles [32]. Angiotensin II generated in the efferent arterioles may act on epithelial cells of tubules of the renal medulla. From preglomerular arterioles and glomerular capillaries, angiotensin II may reach and stimulate mesangial cells, which contract and reduce the glomerular capillary surface area [10]. Angiotensin II was demonstrated by immunohistochemical techniques within juxtaglomerular cells [32], as well as renin, angiotensin I and ACE [23]. ACE activity was demonstrated in the epithelial cells of proximal tubules as well as in the endothelial cells of intrarenal arterioles. Intrarenal release of angiotensin influences the constriction of the efferent arterioles and acts on distal convoluted tubules, permitting the conservation of fluid volume [15]. These data suggest that an intrarenal renin-angiotensin system exists and acts in conjunction with systematically formed angiotensin II.

Aprotinin, a nonspecific inhibitor of renal kallikrein, can reduce renal blood flow in rats, while blood pressure is not modified [17]. Angiotensin II, which is generated near glomeruli in the kidney, specifically stimulates the synthesis of prostacyclin, which in turn may act as a modulator of the intrarenal vascular action of angiotensin II [24].

Testing the specific antibody anti-inhibitors against sections of the human kidney demonstrated a strong inhibitor II-related immunoreactivity at the level of distal convoluted tubules and blood vessels. These findings considered together suggest that the intrarenal kallikrein-kinin system takes part in the renal hemodynamic regulation. In accordance with other authors [33], we believe that locally generated kinins may contribute to the acute vasodilator action of ACE inhibitors.

El-Dahr et al. [3] have proved that differential regulation of renin-angiotensin and kallikrein genes may be an important pathogenetical factor in renovascular hypertension. The renal kallikrein gene, in marked contrast to renin, became downregulated. In other experiments in rats [34], after clipping one renal artery, the renal kallikrein gene expression was not altered suggesting the absence of an enhanced counteracting kinin influence.

The demonstration of an inhibitor II-related immunoreactivity in the tubules and vessels of human kidney, in accordance with the findings of above authors, is a strong evidence that a new inhibitor of kallikrein exists and acts also in humans as an important key in the kallikrein-renin-angiotensin chain. The proved inhibitor in the human kidney may intervene in the modulation of kallikrein-kinin system and thus represent a key role in the intrarenal mechanisms of blood flow and pressure regulation.

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