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Evidence for a stimulatory effect of high potassium diet on renal kallikrein

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Evidence for stimulatory effect of high potassium diet on renal kallikrein. Considerable evidence indicates that the connecting tubule cells, a type of cell of the distal nephron which seems to participate on potassium secretion, may be the place where renal kallikrein is synthetized. As potassium secretion and kallikrein synthesis may occur in the same cells, we studied the effect of high potassium diet on renal kallikrein production. The kallikrein containing cells from rats fed a normal and high potassium diet were evaluated using a combination of morphometric analysis, conventional electron microscopy, and ultrastructural immunocytochemistry. High potassium diet produced hypertrophy and hyperplasia of the kallikrein containing cells. Hyperplasia was sustained by an increased number of immunoreactive cells/mm^2 (151 ± 14 vs. 86.4 ± 12, $P < 0.01$), an increased number of binucleated immunoreactive cells/mm2 (11.90 ± 2.1 vs. 3.77 ± 0.17, $P < 0.005$), and by the presence of mitosis. Cell hypertrophy was sustained by an increased cross–sectional area of immunoreactive cells (μ^2) (320.4 ± 21 vs. 104.5 ± 6.1, $P < 0.001$), by an increased area of basal plasma membrane infoldings, by an hypertrophy of the components of the Golgi complex, hypertrophy of the components of the rough endoplasmic reticulum, and by a larger number of secretory–like vesicles containing kallikrein. The rats fed with high potassium diet had higher values on urinary kallikrein excretion–amidase activity (3.70 ± 0.51 vs. 2.01 ± 0.37 units/day, $P < 0.02$), higher values on potassium excretion (18.8 ± 1.7 vs. 1.31 ± 0.1 mmol/day, $P < 0.001$), and higher urinary volume (51.5 ± 5.3 vs. 12.2 ± 1.6 ml/day, $P < 0.001$). The increased size of the kallikrein containing cells correlated with kallikrein excretion ($r = 0.7013$, $P < 0.002$). These results suggest that high potassium diet stimulates the kallikrein containing cells of the distal nephron producing hyperplasia and hypertrophy. Taken the ultrastructural changes together with the increased urinary excretion of kallikrein, the results suggest that a high potassium diet increased the synthesis and secretion of kallikrein. The nature of this stimulatory effect cannot be elucidated from the present study.

The renal kallikrein–kinin system seems to participate in the control of electrolytes, water excretion and renal vascular resistance [1–4].

The main enzyme of the system, renal kallikrein is a serine protease (EC 3.4.21.35) which releases kinins from its natural substrate kininogen [1–4] and also activates renin [5, 6]. Kinins are able to affect renal function directly or via prostaglandin stimulation [7–9]. The studies on the participation of the renal kallikrein–kinin system on the excretory function of the kidney have been directed mostly to the control of sodium and water excretion [1–4,10] with little attention on the renal metabolism of potassium, a topic in which there are few studies [11].

Using microdissected rat and rabbit nephrons, kallikrein has been found to be restricted to the connecting tubule [12–14]; and by means of the ultrastructural immunocytochemical methods we have described recently, the cellular localization of renal kallikrein as well as its subcellular distribution is in the distal nephron of the rat [15, 16]. Kallikrein is present exclusively in one type of cell of the distal nephron, namely the connecting tubule cell (CNTc). As kallikrein is localized in subcellular organelles involved in the synthesis processing and secretion of glycoproteins, we have postulated that renal kallikrein is synthesized in this type of cell [16].

The CNTc is one of the two types of cells which comprise the connecting tubule and whose ultrastructural features were described few years ago by Crayen and Thoenes [17]. So far, the available indirect evidence obtained from microperfusion, micropuncture or electron microscopy suggests that the CNTc participates at least in part in the secretion of potassium by the distal nephron [18, 19].

In view of the possible co-existence of biosynthesis of kallikrein and secretion of potassium in the same cells, and the knowledge that this type of cell undergoes major ultrastructural changes during chronic high–potassium load [20], we studied the effects of high potassium intake on the urinary excretion of kallikrein and electrolytes, and correlated them with the ultrastructural changes of the kallikrein producing cells of the distal nephron.

Methods

Sheep antiserum against kallikrein and purified rat urinary–kallikrein were from Dr. John J. Pisano of the National Institutes of Health, Bethesda, Maryland, USA. Rabbit antiserum against sheep IgG and normal serum was obtained from Cappel Laboratories, West Chester, Pennsylvania, USA. Goat antiserum against rabbit IgG, normal rabbit serum, normal goat serum and peroxidase–antiperoxidase (PAP) complex of rabbit origin was from Sternberger–Meyer, Jarrestville, Maryland, USA. 3-3′ diaminobenzidine tetrahydrochloride, Tris, and carrageenan was from Sigma, St. Louis, Missouri, USA. Osmiun tetroxide, paraformaldehyde, glutaraldehyde, paraplast–plus, Epon, Araldite was from Ted Pella Inc., Tustin, California, USA.

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Experimental design

Male Holtzman rats (body wt 240 to 280 g) were maintained on tap water and fed ad libitum until the time of study. Two experimental groups were studied: 1) normal potassium diet (N = 8), the rats were fed with a normal diet containing 0.1058 moles of KCl/g and tap water; 2) high potassium diet (N = 8), the rats were fed with normal diet plus a 0.134 M, 0.268 M and 0.402 M KCl solution to drink ad libitum in the first, second and third week, respectively. The rats were placed on individual metabolic cages twice a week for urine collections. At the end of the third week of study the animals were anesthesized, and the kidneys quickly removed and processed as detailed below.

Tissue processing

Fixation and embeddment. The kidney tissue was cut following the cortico-papillary axis in 2 mm thick pieces, including cortex and medulla. The fixative used as well as the embedding media depended on the subsequent use of the material. Thus, for light microscopy, the kidney sections were fixed in Bouin's solution for 24 to 48 hours and embedded in paraplast. For conventional electron microscopy the sections were fixed by immersion in 4% paraformaldyhe plus 2.5% glutaraldehyde in 0.2 M phosphate buffer pH 7.4 for two hours, post-fixed in 1% OsO_4 for two hours at 4°C and embedded in Epon–Araldite. And for ultrastructural immunocytochemistry the tissue sections were fixed in buffered picric acid–paraformaldehyde–glutaraldehyde (Zamboni's fixative plus 1% glutaraldehyde) for four to eight hours at room temperature (22°C) as described previously [15, 16].

Immnostaining

The immunostaining procedure used was the unlabelled antibody method of Sternberger [21, 22] with the modifications we have already used to describe the cellular localization of renal kallikrein as well as its subcellular distribution in the distal nephron of the rat [15, 16].

Light microscopy immunocytochemistry. Paraplast sections (6 μ) were mounted on glass slides and stored. Before the immunocytochemical staining they were deparafinized with xylene and rehydrated through a graded series of alcohol solutions. The tissue sections were rinsed three times (10 min each time) in 0.05 M Tris-HCl buffer pH 7.8. After rinsing the sections, they were incubated with: 1) sheep antiserum against rat urinary kallikrein (1:35,000) for 18 to 24 hours; 2) rabbit antiserum against sheep IgG (1:1,000) for 30 minutes; 3) goat antiserum against rabbit IgG (1:50) for 30 minutes; 4) PAP complex of rabbit origin (1:75) for 30 minutes; and 5) staining with 3-3'diaminobenzidine (0.2%) plus hydrogen peroxide (0.1%). The antisera and PAP complex were diluted in 0.05 M Tris-HCl buffer pH 7.8 containing 0.5% Triton 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 0.05 M Tris-HCl buffer pH 7.8 three times (5 min each time).

Ultrastructural immunocytochemistry. The staining procedure was done in 40 μ cryostat sections of the tissue already fixed with the pre-embedding method for electron microscopy as modified previously by us [15, 16]. Briefly, the incubations steps were similar to those described for light microscopy. An intensification step with 1% osmium tetroxide for two hours at 4°C was added after the diaminobenzidine–hydrogen peroxide step. Finally, the tissues were cut into small pieces (2 × 2 mm) and embedded in the mixture of resins (Epon–Araldite). Thin sections (600 Å) were cut with either a glass or a diamond knives on an ultramicrotome (Ultratome IV, LKB, Stockholm, Sweden), contrasted with lead citrate and examined with an electron Philips EM-300 microscope (Philips, Eindhoven, Holland).

Morphometry

Light microscopy. In order to have a rough estimation of the number and size of the kallikrein–immunoreactive cell population, a simple morphometric analysis of randomized sections from kidneys from both groups was done. The whole population of kallikrein immunoreactive cells was estimated by counting all the kallikrein immunoreactive cells with nuclear profile in each tissue section. The estimation was done in 6 μ thick paraplast sections immunostained for kallikrein and counterstained with hematoxylin by using light microscopy (Leitz) at a magnification of × 500. The cells were counted in tissue sections from six control rats and six rats from the potassium group. One section (including the whole kidney section) was used for each rat and approximately 3,500 and 5,000 immunostained cells were counted in each section from control and potassium groups, respectively.

To calculate the area of the whole kidney section, the area of cortex and the area of medulla, a photographic enlargement (× 20) was done and the areas was measured using a planimeter (OTT, Kempten, Bayern, Germany). As the kallikrein immunoreactive cells were located exclusively in the cortex, they were expressed as cells per mm^2 of cortex area.

To calculate the percentage of kallikrein immunoreactive cells in relation to the whole cortical–cell population, the latter was estimated by counting the cells from the cortex using a light microscope with a graticule in the eyepiece at a magnification of × 500. At this magnification the graticule gives an area of 0.0277 mm^2. Twenty, randomly chosen 0.0277 mm^2 areas were examined in each section, and all the cells with nuclei were counted. With this information the number of cortical cells was further calculated per mm^2.

Electron microscopy. The cross–sectional area of randomly–chosen immunoreactive cells from both groups (N = 24) was measured using a planimeter and expressed in μ^2. For this purpose cells cut in cross–section with an intact basal lamina and two visible zonula occludens were examined at a final printed magnification of × 4,000.

Urinary kallikrein

Kallikrein activity was estimated by its amidolytic activity on the chromogenic tripeptide sustrate, H-D-Val-Leu-Arg-p-nitroanilide, S-2266 (Kabi Diagnostica, Stockholm, Sweden) as described by Amundsen et al [23] and modified by Bonner and Marín–Grez [24]. Each urine sample was assayed against its own blank containing aprotinin.

Kallikrein activity was expressed as kallikrein amidolytic activity (KAU); being one unit, the enzymatic activity was able to hydrolize one μmole of substrate per minute at 37°C, pH 8.2. No activation was done with trypsin, therefore the activity represents only active kallikrein.

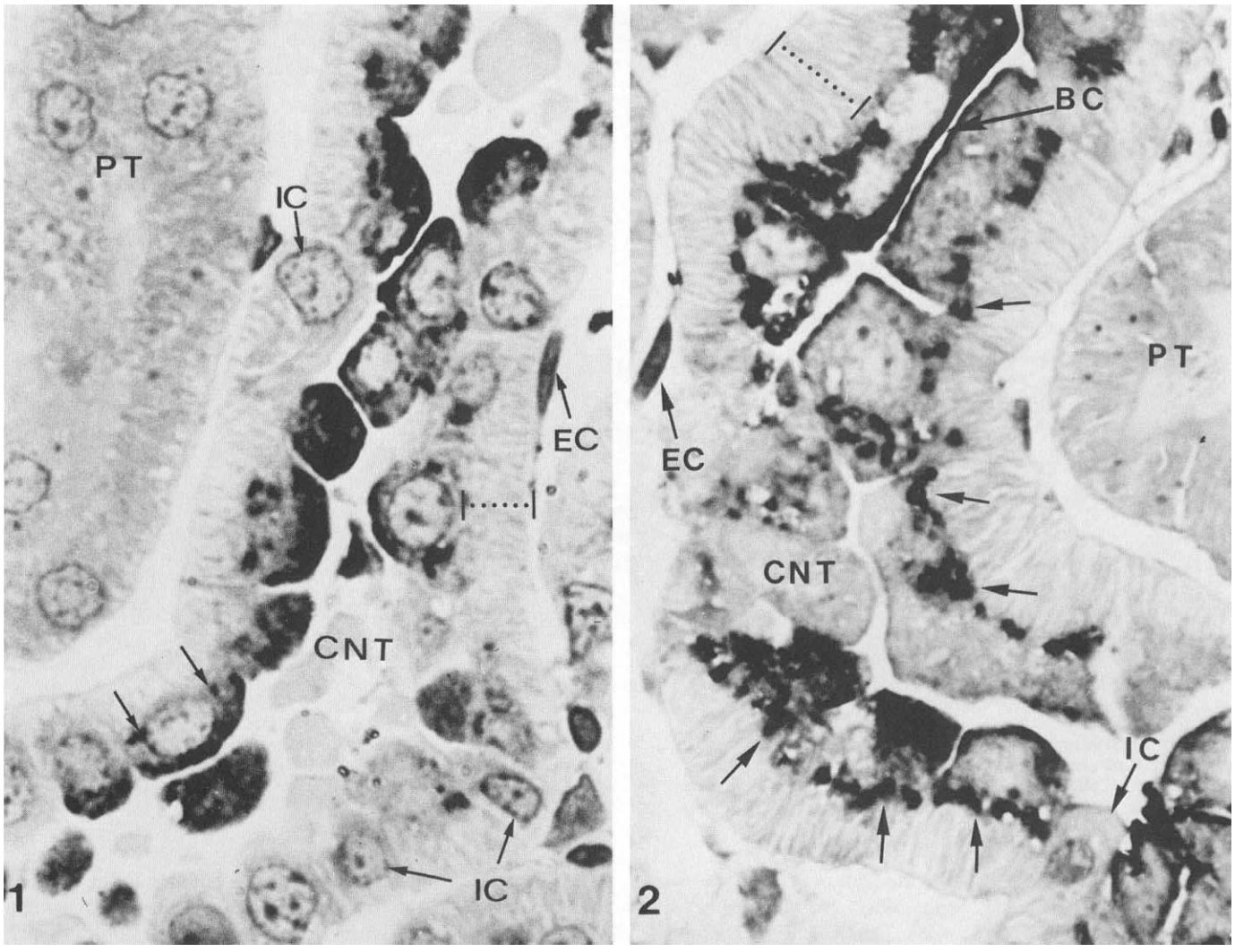

Fig. 1. *Light micrograph showing connecting tubule (CNT) from a control animal.* Kallikrein immunostaining is present in the connecting tubule cells concentrated in the apical pole and the perinuclear area. Arrows point to the Golgi area. No staining is observed on either the proximal tubule (PT) or intercalated cells (IC); (EC) is the nuclei of an endothelial cell. The size of the basal part of the cell comprised of plasma membrane infoldings and mitochondria is marked by a dotted line (× 1,600).

Fig. 2. *Light micrograph showing connecting tubule (CNT) from an animal on a high potassium diet.* An overall hypertrophy of the kallikrein–containing cells is clearly observed when compared with Figure 1. There is an absence of changes in size in either the intercalated cells (IC), proximal tubule (PT) or the nuclei of the endothelial cell (EC). Note the hypertrophy of the basal portion of the cell, where membrane infoldings and mitochrondia (dotted line) are located, and the marked hypertrophy of the immunoreactive Golgi complex, which occupies almost all the area below the nuclei (arrows). A large binucleated cell containing kallikrein can be observed (BC) (× 1,600).

Electrolytes

Sodium and potassium concentrations were measured in urine samples using a flame emission spectrophotometer (Perkin Elmer, USA).

Statistics

The normal distribution for each variable was established by means of a Kolmogorov–Smirnov test. A Pearson's correlation test was done to study the correlation between variables. Student's *t*-test for unpaired data was performed to analyze the difference between the means. All data are expressed as means ± SE. A P value < 0.05 was considered as significant. All the statistical analysis was done in a Digital 2020 computer using the SPSS program [25].

Results

Kallikrein immunostaining was observed exclusively over the connecting tubule cells (CNTc) of the distal nephron regardless of the type of potassium diet the rats received (Figs. 1 and 2). When the kallikrein immunoreactive cells from rats from both groups were compared, using high resolution light microscopy (1 μ thick sections), those from rats fed with a high potassium diet were consistently larger than those from rats fed with a normal diet (Figs. 1 and 2).

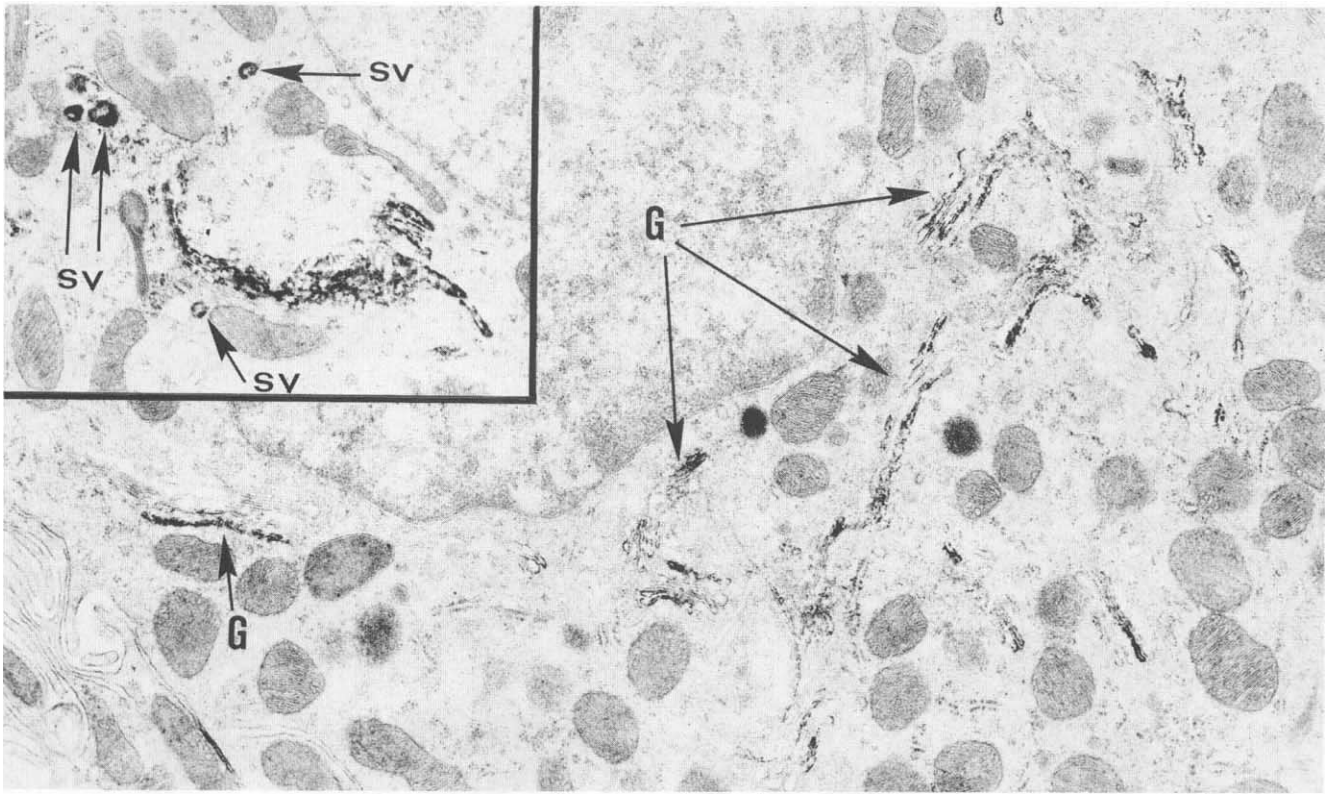

Fig. 3. *Perinuclear area of a connecting tubule cell immunostained for kallikrein from an animal on a high potassium diet.* A hypertrophied Golgi complex (G) containing kallikrein is observed with large number os stacks containing kallikrein (× 18,500). In the bottom left corner of the Figure, a portion of a cell basal plasma membrane infolding immunostained for kallikrein can be seen *Inset*. An immunoreactive Golgi stack and secretory–like vesicles (sv) arising from the Golgi *trans* face (× 17,500).

The immunoreactive cells from the high potassium diet were consistently hypertrophied, some cells were binucleated, and mitosis was present [Figs. 3, 4, 5]. This cell hypertrophy was confirmed by estimating the cell size by measuring its cross–sectional area, which almost tripled the size of the cells from the control group (320.4 ± 21 μ^2 vs. 104.5 ± 6.1 μ^2, $N = 24$, $P < 0.001$). The hypertrophy was in part due to the increase in the area of basal plasma–membrane infoldings. In addition there was an increase in the cytoplasmic area, hypertrophy of the components of the Golgi complex, hypertrophy of the rough endoplasmic reticulum (RER) and a large number of secretory–like vesicles (Figs. 2, 3, 4, 6).

The population of kallikrein immunoreactive cells was also different on rats fed on a high potassium diet when compared with those from the control diet group. An increase was observed in the number of immunoreactive cells per mm^2 (151 ± 14 vs. 86.4 ± 12, $P < 0.005$). There was also an increase in the number of binucleated immunoreactive cells (11.90 ± 2.1 vs. 3.77 ± 0.17, $P < 0.005$), as well as the presence of mitoses which still had traces of kallikrein (Table 1, Fig. 5).

Besides the changes in size and number of the kallikrein immunoreactive cells from the high potassium diet, a different pattern of intracellular staining was observed by light microscopy. The cells from rats fed on a normal diet had kallikrein immunostaining concentrated in the luminal side with little staining in the perinuclear area below the nuclei, where the Golgi apparatus reside (Fig. 1). The cells from rats fed on a high potassium diet displayed a more intense staining in the luminal side together with an extensive staining below the nuclei, pointing at light microscopic level to the hypertrophy of the components of the Golgi complex (Figs. 2 and 4). This observation was confirmed with ultrastructural immunocytochemistry, which revealed a hypertrophied Golgi apparatus with dense staining for kallikrein over the cisterna of a large number of Golgi stacks and over numerous vesicles arising from the Golgi *trans* face (Fig. 3).

A more detailed observation of the ultrastructure by conventional electron microscopy revealed an increase in the number and size of the Golgi components (many Golgi stacks had dilated ends and numerous vesicles arising from the ends), as well as increased number of cisterna of the RER distributed all over the cells, which contrast with cells from control rats where the RER was rather scarce (Fig. 4).

On the other hand, the more intense kallikrein immunostaining observed in the luminal pole of the cells from rats fed on a high potassium diet was due to a large number of vesicles containing kallikrein close to that portion of the cells (Fig. 6). This increase in the number of vesicles was observed by conventional electron microscopy as well (Fig. 4). Although immunostaining was seen over the basal cell, plasma membrane infoldings (Fig. 3) no visible differences were observed between groups.

No changes were observed in other cells of the connecting tubule, namely the intercalated cells (Figs. 1, 2, 4, 5).

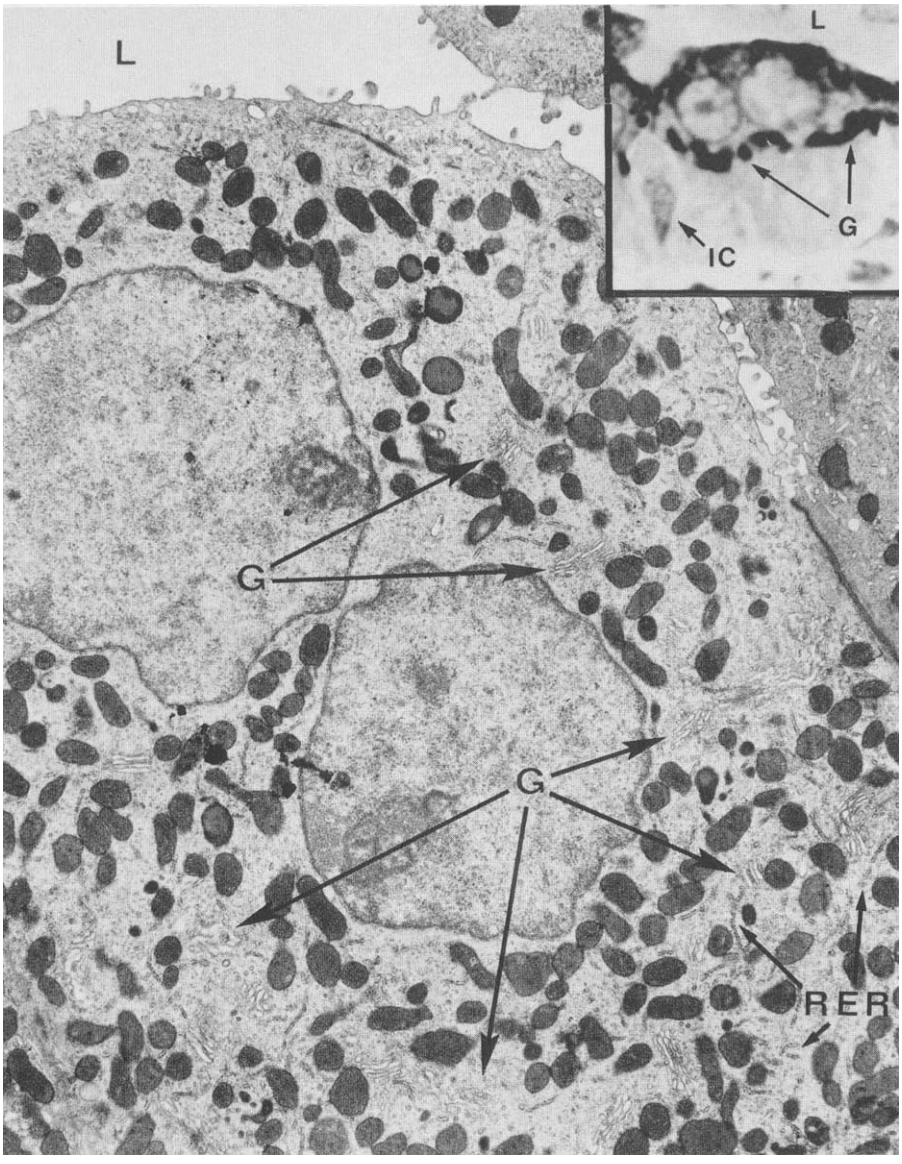

Fig. 4. *Conventional electron micrograph of a binucleated connecting tubule cell from an animal on a high potassium diet.* The cell has a marked hypertrophy of both the Golgi complex (G) and the rough endoplasmic reticulum (RER). Numerous components of the Golgi complex and RER are located in the perinuclear area reaching the most apical portions of the cell. L = tubular lumen (8,200). *Inset*: Light micrograph of an immunostained binucleated–connecting tubule cell from an animal on a high potassium diet. Note the abundant immunoreactive kallikrein in the Golgi area (G), and in the apical portion of the cell. An intercalated cell (IC) is present between two immunoreactive cells (× 1,400).

Urinary kallikrein, water and electrolyte excretion

Table 2 summarizes the results on urinary kallikrein, water, sodium and potassium excretion. Significant differences were observed between groups on urinary kallikrein, water and potassium excretion. The rats fed with high potassium diet had higher values on urinary kallikrein excretion (3.70 ± 0.51 vs. 2.01 ± 0.37, $P < 0.01$), potassium excretion (18.8 ± 1.7 vs. 1.31 ± 0.1, $P < 0.001$) and water excretion (51.5 ± 5.3 vs. 12.2 ± 1.6, $P < 0.001$). No difference was observed between groups on sodium excretion.

Urinary kallikrein excretion correlated with potassium excretion ($r = 0.5360$, $P < 0.02$) and with water excretion ($r = 0.5063$, $P < 0.03$). No correlation was observed between urinary kallikrein and sodium excretion ($r = 0.1868$, NS).

Morfofunctional correlation

As suggested functions for the CNTc are kallikrein synthesis and secretion, we performed a correlation between the estimated size of the cells—measured by its cross sectional area—with urinary kallikrein excretion. The size of kallikrein immunoreactive cells correlated well with urinary kallikrein excretion ($r = 0.7013$, $P < 0.002$).

Discussion

The present study revealed that high potassium intake stimulated renal kallikrein production, since hypertrophy and hyperplasia of the kallikrein producing cells with an increased urinary excretion of the enzyme was found. The hyperplasia observed in rats on a high potassium diet is supported by the presence of an increased number of immunoreactive cells, by the increased number of binucleated immunoreactive cells[1], and by the presence of mitosis containing traces of im-

[1] At present neither the origin nor the meaning of the binucleated cells is clear; phenomena such as cell fusions or failure of cytoplasmic cleavage at late telophase have been discussed in the past with reference to liver cells. An increase in the number of binucleated cells under constant stimuli has been observed, which is a situation in which the cell proliferation increases and the mitotic index is high [26].

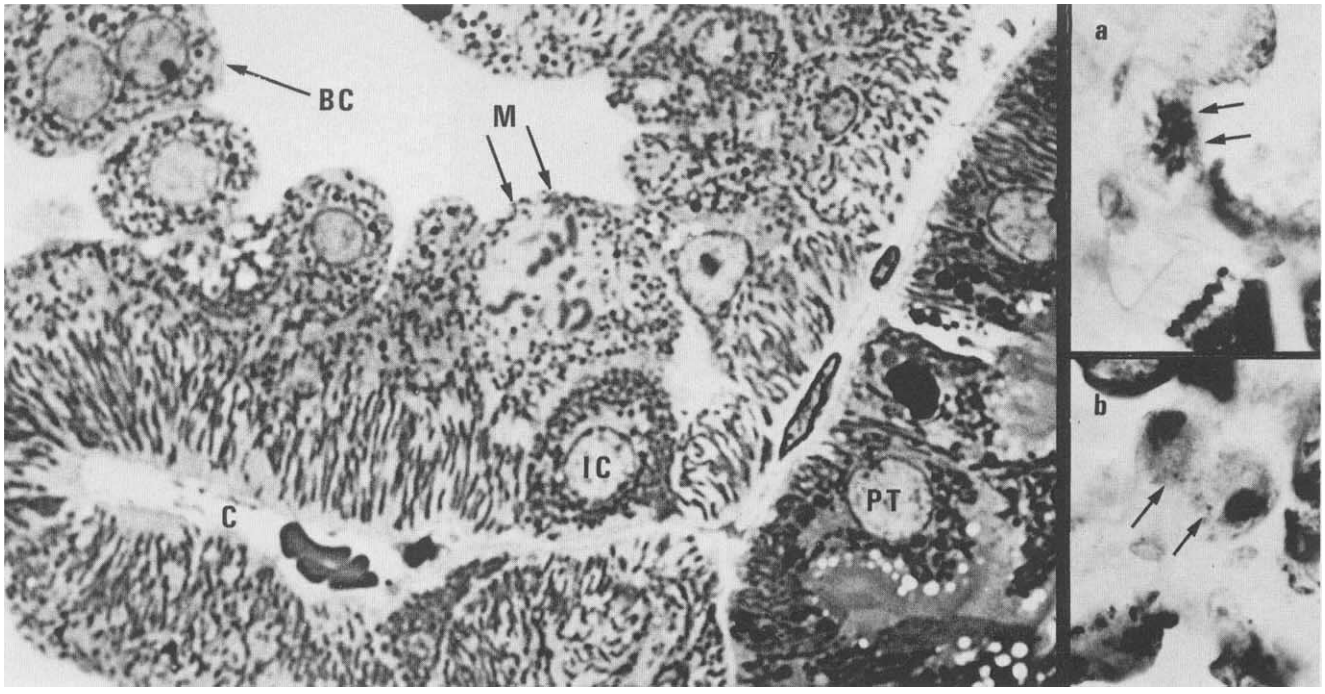

Fig. 5. *High resolution light micrograph of a connecting tubule from an animal on a high potassium diet.* (1.0 μ thick section, Epon–Araldite). Note the hypertrophy of the connecting tubule cells when compared with the intercalated cells (IC) and proximal tubule cells (PT), the presence of a mitosis (M) and a binucleated cell (B C). (C) is a capillary vessel containing eritrocytes (× 1,600). *Insets*: Paraplast sections immunostained for kallikrein counterstained with hematoxylin from animals on a high potassium diet. Both insets show mitosis among immunoreactive cells. A prophase and an immunoreactive telophase is observed in insets a and b, respectively. (× 900).

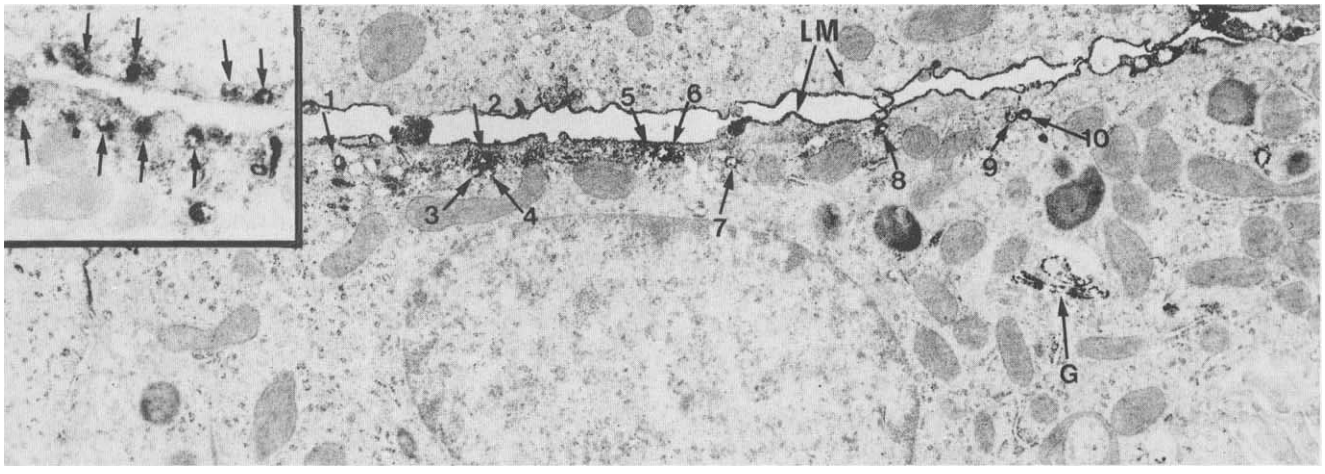

Fig. 6. *Apical portion of a connecting tubule cell immunostained for kallikrein from an animal on a high potassium diet.* A large number of kallikrein containing vesicles (numbered from 1 to 10) are observed close to the luminal plasma membrane (LM), (G) is the Golgi complex. (× 11,400). *Inset*: Higher mangnification of the kallikrein containing vesicles (arrows) in close proximity to the LM. The vesicles are similar to those arising from the *trans* face of the Golgi complex observed in Fig. 3. (× 18,000).

munoreactive kallikrein (no mitosis were observed in control rats). It should be pointed out that the presence of kallikrein in cells in mitosis was observed in thick section (6 μ paraplast sections) and kallikrein was uniformly distributed over the periphery of the cells. Since one can anticipate that no synthesis of kallikrein occurs during the mitotic phases, the presence of kallikrein most likely represent remmant kallikrein already synthesized before the cell entered into division.

The hypertrophy of the kallikrein producing cells (CNTc) from rats on a high potassium diet was consistently observed. A detailed analysis of the hypertrophied CNTc revealed hypertrophy of the basal plasma–membrane infoldings as well

Table 1. Population of kallikrein–immunoreactive (KI) cells in rats on normal and high potassium diet

	KI cells *cells/cortex area*	KI cells *cells/mm²*	KI binucleated *cells/mm²*	Mitosis *mitosis/mm²*
Normal potassium diet	3,255 ± 145[a]	86.4 ± 12	3.77 ± 0.17	0
High potassium diet	5,085 ± 342[a]	151 ± 14	11.90 ± 2.1	0.18 ± 0.04[b]
$N = 6$	$P < 0.005$	$P < 0.01$	$P < 0.005$	

Values are means ± SEM.

[a] The population of kallikrein–immunoreactive cells in normal diet and high potassium diet groups represent 1.7% and 2.8%, respectively, of the whole cortical cell population.

[b] The mitosis displayed little kallikrein immunostaining and was primarily located in the cell periphery.

as a marked overall hypertrophy of the biosynthetic–secretory apparatus (RER, Golgi, secretory like vesicles).

The hypertrophy of the basal cell portion has been reported by others and is associated with an increase in basal membrane area [20, 27, 28]. It is well established that an increase in Na-K-ATPase activity plays an important role in mediating the secretion of potassium by cells of the distal nephron during chronic potassium load [29], and provided that the number of pump sites per unit of membrane area remains constant, chronic changes in transport capacity are reflected by corresponding changes in membrane area [27, 30].

The hypertrophy of the Golgi complex, RER, and the increased number of secretory vesicles indicate an stimulated biosynthetic activity occurring on the kallikrein producing cells. Although part of this hypertrophy could be devoted to the synthesis of plasmalemmal proteins, like the Na-K-ATPase which consist of a transmembrane catalytic subunit (100,000 daltons) and an associated glycoprotein (45,000 daltons), the presence of an extensive immunoreaction for kallikrein over the cisternal space of the Golgi apparatus and over secretory–like vesicles indicates that the hypertrophy is due to the increase in the synthesis and intracellular processing of kallikrein.

The increased number of secretory vesicles containing kallikrein close to the luminal space of the CNTc suggests an increased excretion of kallikrein into the tubular space. But, it could be argued that the increased number of vesicles in the apical portion of the cell is due to a reduction in the rate of vesicle fusion with the apical membrane causing the accumulation of vesicles, as it is nearly impossible to identify the nature of a dynamic event by sampling one point in time by electron microscopy. However, as the urinary excretion of kallikrein is increased as well, we tend to believe that the increased number of vesicles means increased excretion of the enzyme. This hypothesis receives further support with the correlation coefficients obtained between the estimated cell size of the CNTc and the urinary excretion of kallikrein ($r = 0.7013$, $P < 0.002$).

The nature of this stimulatory effect of high potassium intake on renal kallikrein cannot be elucidated from the present study because both renal kallikrein and renal potassium excretion are under a multihormonal regulation [31], and high potassium intake could stimulate renal kallikrein acting via mineralocorticoids, glucocorticoids, prostaglandins or even directly.

Although there is little doubt on the stimulatory effect of aldosterone on renal kallikrein, its mechanism is still unknown. Early studies suggested the existence of a direct effect on the kallikrein producing cells [32]; more recent work from the same authors have postulated an indirect stimulatory effect of aldosterone on renal kallikrein [33]. The participation of glucocorticoids could not be excluded as they are kaliuretic, [34] produce hypertrophy of cortical collecting tubule cells (cells which may correspond to CNTc, since early studies included the CNT in the late distal convoluted tubule and initial collecting tubule) [35], and can increase renal kallikrein [36, 37].

Table 2. Urinary kallikrein, water, sodium and potassium excretion during normal and high potassium intake

	UV *ml/day*	$U_{Na}V$ *mmol/day*	U_KV *mmol/day*	$U_{Kall}V$ *U/day*
Normal potassium diet	12.2 ± 1.6	1.94 ± 0.2	1.31 ± 0.1	2.01 ± 0.37
High potassium diet	51.5 ± 5.3	2.35 ± 0.2	18.8 ± 1.7	3.70 ± 0.51
$N = 8$	$P < 0.001$	NS	$P < 0.001$	$P < 0.02$

Abbreviations are: UV, urinary volume; $U_{Na}V$, urinary sodium excretion; U_KV, urinary potassium excretion; $U_{Kall}V$, urinary kallikrein excretion. Values are means ± SEM.

On the other hand, the studies of Marchetti et al [14] have provided convincing evidence showing that DOCA treatment increased active kallikrein in CNT and in urine, although they believe that the long term effect of DOCA on renal kallikrein probably results from an indirect effect.

Prostaglandins might be mediators of this effect as well, since a prostaglandin mediated mechanism has been postulated for kallikrein release [38, 39]; moreover, Nasjletti et al has reported recently that high potassium intake selectively stimulates renal PGF2α production associated with an increase on urinary kallikrein as well [40].

In summary, we have described the stimulatory effect of high potassium diet on urinary kallikrein excretion. These results, taken together with the cellular changes produced on the kallikrein–immunoreactive cells observed with light and ultrastructural immuncytochemistry and conventional electron microscopy, suggest that high potassium intake stimulates the synthesis and release of renal kallikrein by the CNTc. Although we discuss some of the possible mechanisms mediating this stimulatory effect on renal kallikrein, we cannot draw conclusions from other literature or from our own results of the exact nature of this effect. Studies ongoing in our laboratory are directed to further elucidate this point.

Acknowledgments

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