ABSTRACT—Renal kallikrein is one of the tissue kallikreins, and the distal nephron is fully equipped as an element of the kallikrein-kinin system. Although a low excretion of urinary kallikrein has been reported in essential hypertension, the results from studies on patients with hypertension are not consistent. Congenitally hypertensive animals also excrete lowered levels of urinary kallikrein, but the effects of this are yet unknown. Extensive genetic and environmental studies on large Utah pedigrees suggest that the causes of hypertension are closely related to the combination of low kallikrein excretion and the potassium intake. Mutant kininogen-deficient Brown Norway-Katholiek rats, which cannot generate kinin in the urine, are very sensitive to salt loading and to sodium retention by aldosterone released by a non-pressor dose of angiotensin II, which results in hypertension. The major function of renal kallikrein-kinin system is to excrete sodium and water when excess sodium is present in the body. Failure of this function causes accumulation of sodium in the cerebrospinal fluid and erythrocytes, and probably in the vascular smooth muscle, which become sensitive to vasoconstrictors. We hypothesize that impaired function of the renal kallikrein-kinin system may play a pivotal role in the early development of hypertension. Inhibitors of kinin degradation in renal tubules and agents, which accelerate the secretion of urinary kallikrein from the connecting tubules and increase the generation of urinary kinin, may be novel drugs against hypertension.

Keywords: Renal kallikrein, Kininogen, Urinary kinin, Sodium accumulation, Hypertension
Introduction

The primary cause of essential hypertension has not been identified despite intensive research on the various mechanisms that may be involved in its development. The hypothetical possibility that hypertension results from either an excess of vasoconstrictive substances or a deficiency of vasodilating substances has led to research into the roles of the renin-angiotensin system and the kallikrein-kinin system.

The genetic and environmental determinants of hypertension, lipid abnormalities and coronary artery disease have been studied for 15 years in Utah in population-based multigenerational pedigrees (1). According to this study and related studies, the genetic loci for the structural genes for renin (2) and angiotensin-converting enzyme (ACE) (3) and the sodium antiport system (4) were not found to be DNA markers for hypertension. Angiotensinogen shows moderate hypertension susceptibility, and the angiotensinogen variant, recently found to be a promoter of hypertension, is present in approximately 30% of the general population (1, 5). Another recent review (6) indicates that the genes of the renin-angiotensin-aldosterone system are directly responsible for some types of hypertension such as Liddle's syndrome, but in familial essential hypertension, neither renin nor ACE genes contribute to a large extent to the genetics of hypertension, at least in humans. An ACE gene polymorphism may be a strong marker of coronary and cardiac diseases and diabetic complications. Angiotensinogen gene polymorphism appears to be linked to hypertension, and molecular variants of this protein are associated with high blood pressure in various populations and ethnic groups. An angiotensin II AT_{1}-receptor variant is associated with essential hypertension, and this gene variant together with ACE gene polymorphism increases the relative risk of myocardial infarction. Mice that are made completely deficient in the angiotensinogen gene by a gene-targeting method cannot maintain their normal systemic blood pressure and they die gradually after birth (7). In contrast, when the angiotensin II type 1a-receptor gene is made deficient in mice by a gene-targeting method, they also cannot maintain normal systemic blood pressure, despite having a markedly high renin activity in their plasma, but no death after birth was observed (8). The difference in the death rate after birth between the two types of deficient in the components of the renin-angiotensin system may be due to residual secretion of aldosterone in the latter mutant mice through angiotensin II type II receptors present in the adrenal glands, whereas the former may fail to secrete aldosterone, because they lack the ability to generate angiotensin II. Therefore, the important role of the renin-angiotensin-aldosterone system in maintenance of the normal systemic blood pressure through aldosterone release might have been clarified.

In contrast, segregating single-gene effects were found for several "intermediate phenotypes" associated with hypertension, including erythrocyte sodium-lithium countertransport (9), intra-erythrocytic sodium levels (10) and total urinary kallikrein excretion (11). Furthermore, an important gene-environment interaction was found between urinary kallikrein and potassium intake (1, 12). These studies on the genetic determinants of hypertension indicate that the renal kallikrein-kinin system may play an important role in the development of hypertension. Many reviews on the renal kallikrein-kinin system have been published (13–17). A more recent review on the roles of the kallikrein-kinin system in human diseases, particularly in hypertension, was published in 1995 (18). A good review on the kallikrein-kinin system, acting locally in endothelial cells, cardiac myocytes and vascular smooth muscles, and on its roles in ventricular hypertrophy, myocardial ischemia and remodeling will soon be published (19). The transgenic mice bearing an overexpressed human kallikrein have high levels of kallikrein in their serum and various tissues, which results in sustained hypotension (20). Mice whose bradykinin B_{2}-receptor gene was knocked out were reported (21), but induction of hypertension has not been reported. Despite recent accumulation of ample data, the definitive role of the renal kallikrein-kinin system in essential hypertension remains to be clarified, because it is difficult to completely eliminate the components of this system in a living animal. We have been studying the role of the renal kallikrein-kinin system with mutant kininogen-deficient rats that cannot generate kinin in their urine (Brown Norway-Katholiek (BN-Ka) rats). The susceptibility of these mutant kininogen-deficient BN-Ka rats to salt and development of hypertension have been summarized in a short review (22).

The present review will discuss the mechanisms of the development of essential hypertension with particular reference to the renal kallikrein-kinin system.

I. Renal kallikrein-kinin system

1. The kallikrein-kinin system: general points

Bradykinin (BK) is a biological peptide with potent activities in vasodilatation, increased vascular permeability, smooth muscle contraction, pain generation, natriuresis, diuresis and renal blood flow increase. This peptide is released from precursor proteins, the kininogens, by proteolytic enzymes, the kallikreins. There are two kallikreins, plasma kallikrein and tissue (glandular) kallikrein, and two kininogens, high and low molecular
weight (HMW and LMW) kininogens. As shown in Fig. 1, plasma kallikrein is present in plasma in its inactive form, prekallikrein, which is directly activated by blood clotting factor XIIa (23), and active plasma kallikrein cleaves BK from HMW kininogen; On the other hand, tissue kallikrein is released in its active form from glandular tissues and the kidney and cleaves lysyl-BK (kallidin) (human) (24) or BK (rat) (25) preferentially from LMW kininogen. Intravascular activation of the plasma kallikrein-kinin system triggers hypotension, while the activation of this system in the perivascular space causes inflammatory responses. The plasma kallikrein-kinin system works independently from the tissue or glandular kallikrein-kinin system in vivo. Plasma kallikrein is inhibited by a soy bean trypsin inhibitor, whereas tissue kallikrein is not, but aprotinin inhibits both kallikreins.

Renal kallikrein is a tissue or glandular kallikrein. The gene of murine tissue kallikrein belongs to a multigene family of similar serine-proteases. Thirteen genes of serine proteases are localized on one chromosome in the rat (26). The true tissue kallikrein gene in the kidney is composed of 5 exons and 4 introns, its length being about 4.5 kilobase pairs (26, 27); and recently, the nomenclature of the glandular kallikrein gene family has been unified (28). The functions of this group of serine proteases are not completely known, but the amino acid sequences of the kallikrein gene family are mutually quite similar, and the substrate specificity and the reactivity against inhibitors or antibodies are shared. The kallikrein-like proteases purified from rat salivary glands include glandular (tissue) kallikrein (rK1), tonin (rK2), rK7, rK8, rK9 and rK10. In the rat kidney, rK1 and rK7 are the main proteases expressed (27). In humans, three serine protease genes, hK1 (glandular kallikrein), hK3 (prostate specific antigen) and hK2, are present on chromosome 19 (29).

2. Angiotensin-converting enzyme inhibitors and bradykinin

Kinin is not constantly generated in the plasma by the plasma kallikrein-kinin system, since plasma kallikrein is present in plasma in an inactive form, prekallikrein, as mentioned above. Plasma prekallikrein is activated only when coagulation factor XII is activated to XIIa, which directly activates plasma prekallikrein. The activation of factor XII is induced by exposure of plasma protein to negatively charged surfaces, such as kaolin, glass, ellagic acid, lipopolysaccharides and carrageenin (30–32). Bacterial proteases liberate BK by activating the factor XII-prekallikrein cascade, or directly, by their proteolytic activity, from guinea pig HMW kininogen (33–36). Plasma prekallikrein is not activated even when plasma is exuded into the perivascular space (37). This was successfully demonstrated when a degradation product of BK, BK-(1-5) or des-Phe8,Arg9-BK, instead of BK itself, was measured in the rat pleural exudate after intrapleural injection of histamine (38). Intrapleural injection of histamine to rats caused exudation of plasma proteins into the pleural cavity, but neither BK-(1-5), des-Phe8,Arg9-BK nor BK was detected (37), whereas intrapleural injection of carrageenin generates a large amount of BK-(1-5) in the exudate, since carrageenin activates factor XII in the plasma proteins (38).

Immunoreactive glandular kallikrein may be present in the plasma (39–42). However, the active kallikrein is
immediately bound to the large amounts of inhibitors present in the plasma (42) and is inactivated, although there have been reports that blood kinins may be generated (43, 44). Therefore, even if kinin is generated in the plasma without activation of factor XII, its amount may be negligible.

The anti-hypertensive effect of ACE inhibitors may be relevant to the inhibition of kininase II or to increased levels of kinin in the plasma. The hypotensive effects of an ACE inhibitor, perindopril, in spontaneously hypertensive rats (SHR) on low- and high-NaCl diets are attenuated by a BK B2-receptor antagonist Hoe 140 (D-Arg³,Hyp³,Thi⁵,Tic⁷,Oic⁸)BK (45). In healthy subjects, plasma kinin levels are increased from 16.1 ± 1.9 pmol/l to 22.4 ± 2.8 or 29.1 ± 4.7 pmol/l (46) after administration of ACE inhibitors. In rats, captopril slightly (from 10±3 to 29±7 pg/ml) increases the BK level in the arterial blood of anesthetized rats (47) (Fig. 2), but this increase in BK is not sufficient to reduce the systemic blood pressure, since an intravenous infusion of nearly 1000 ng/min of BK is required to decrease the systemic blood pressure, and the BK concentration in the arterial blood during the infusion of 1000 ng/min of exogenous BK is 900–1000 pg/ml (47). Therefore, in the anesthetized rats, the concentration of BK in the arterial blood, which is required for reduction of the systemic blood pressure, is 30 times higher than those reached after captopril treatment without infusion of exogenous BK.

Nevertheless, cardiac tissue and endothelial cells contain a local kallikrein-kinin system and the beneficial effects of ACE inhibitors on cardiovascular diseases have been reported (48). The effects of an ACE inhibitor such as ramipril, given in non-blood pressure-lowering doses, are as follows: accumulation of cGMP in cultured bovine aortic endothelial cells; restoration of the increased height of contraction by norepinephrine of isolated aortic rings from rabbits fed a cholesterol-enriched diet and loss of relaxation by acetylcholine of the same aortic rings; inhibition of ventricular fibrillation of isolated working rat heart with postischemic-reperfusion; reduction in infarct size in the rabbit; restoration of the increased left ventricular weight in rats with aortic banding; reduction of neointima formation in rats using a balloon catheter; reduction of myocardial left ventricular hypertrophy in SHR and so forth. Most of the beneficial effects of ACE inhibitors like ramipril were reversed by pretreatment with Hoe 140, and these beneficial effects may be related to the formation of nitric oxide and prostacyclin enhanced by BK released (48).

3. Independent functions of the renal kallikrein-kinin system

The kidney displays a full set of kallikrein-kinin system components in its distal tubules, as shown in Fig. 3. This system works independently from that in other organs and tissues including plasma. The localization of the components of the kallikrein-kinin system in the kidney is summarized in a review (17).
a. Kallikrein

i) Localization: A suspension of rat renal cortical cells contains kallikrein activity (49). Using a single nephron, it was found that more than 85% of the active and inactive kallikreins in the rat kidney are localized in the granular portion of the distal tubule and the cortical collecting duct (50–52). No kallikrein was detected in the glomerulus, the thick ascending limb of Henle’s loop, the bright portion of the distal tubules (macula densa) and the light portion of the cortical collecting tubules (51). Electron micrographic studies indicated that kallikrein was only located in the distal tubules (53, 54). Recent studies confirmed that kallikrein is present exclusively in the granular cells of the connecting tubule of the distal nephron, where kallikrein is concentrated mainly on the luminal side of the cells and at both sides of the nuclei, and to a lesser extent was associated with the plasma membranes and basolateral infoldings. The immunoreactivity is related to free polyribosomes, the rough endoplasmic reticulum and Golgi complexes, suggesting that kallikrein is actively synthesized in this particular type of cell (55–57).

Tissue kallikrein mRNA is expressed dominantly in cells of the distal tubules, but also in the vascular pole of the glomeruli (58) and in the connecting tubules of the outer cortex (59). Although kallikrein is present in the granular peripolar cells of the human kidney, mRNA was not found there (60). It is possible that the kallikrein in these cells has been absorbed from the glomerular filtrate. Tissue kallikrein mRNA and protein are present in the walls of the renal blood vessels (60). From studies with human tissue kallikrein mRNA in diseased kidneys, it was suggested that the tissue kallikrein gene in the kidney may not be constitutively expressed, but is expressed in response to physiological and pathological stimuli; however, this conclusion needs to be confirmed.

ii) Stimuli for kallikrein secretion: Renal perfusion pressure may be one of the major factors controlling urinary kallikrein excretion in anesthetized dogs (61). Chronic arterial constriction of the kidney in conscious dogs and anesthetized rats is associated with a lower kallikrein excretion from the stenotic kidney than from the contralateral kidney (62). In isolated perfused hog and rat kidneys also, kallikrein excretion is dependent on the perfusion pressure (63–65).

A low-sodium diet or salt deprivation always accelerates renal kallikrein synthesis and excretion in humans (66, 67) and rats (68, 69). A study with microdissected segments of rabbit nephron revealed (70) that both active and inactive kallikrein in the granular portion of the distal convoluted tubules and in the cortical collecting tubules (connecting tubules) increased markedly during low sodium intake without altering either the distribution profile or the ratio of active to total kallikrein in the nephron or urine. The increased kallikrein excretion due to prolonged sodium deprivation may be mediated by aldosterone release through activation of the renin-angiotensin system by long-term restriction of sodium intake, since this increase is reversed by the aldosterone antagonist spironolactone and is induced by administration of fluorocortisone, a synthetic sodium retaining steroid (66, 67). Patients with hyper-aldosteronism excrete higher amounts of kallikrein in the urine (67, 71), as Fig. 4 shows. The same phenomenon was seen in patients with Bartter’s syndrome (72). Administration of spironolactone to patients with primary aldosteronism decreases
the high urinary kallikrein level (66, 67). The removal of aldosterone-producing tumors reverses the increased excretion of urinary kallikrein (73).

Long-term administration of deoxycorticosterone is reported to increase kallikrein excretion (74). As Fig. 5 indicates, in deoxycorticosterone acetate (DOCA)-salt hypertension in rats, urinary levels of kallikrein and prokallikrein rose to a peak at the age of 10 weeks (3 weeks after the start of treatment), simultaneously with peaks of urinary excretion of sodium and water in normal Brown Norway-Kitasato rats. However, the increases were transient and the predadministration levels were regained when the systolic blood pressure reached a plateau, at 15 weeks (Figs. 5 and 16) (74) (see below for details).

Accelerated synthesis of kallikrein by aldosterone was reported in isolated rat renal cortex cells in suspension, and the increase of kallikrein was inhibited by spironolactone (49). Aldosterone also increases kallikrein release from rat renal cortical cell plasma membranes and endoplasmic reticulum (75). Adrenalectomy decreased both the kallikrein content in the connecting tubules and the Na⁺/K⁺ ATPase activity in microdissected rabbit nephron, but a single injection of aldosterone to adrenalectomized rats caused restoration of the Na⁺/K⁺ ATPase activity, although not the kallikrein content (76).

The effects of high sodium intake are still controversial. Acute sodium loading in rats induced an increase in urinary kallikrein excretion, but a second administration of sodium after 40-min interval did not enhance the kallikrein concentrations in urine (77). Furthermore, feeding rats a high-salt diet for 10 days decreased the total amount of immunoreactive kallikrein in the urine and kidney (78).

Excretion of urinary kallikrein varies directly with potassium intake and parallels the excretion of aldosterone without increased excretion of sodium in both normal and hypertensive subjects. The increase brought about in urinary kallikrein excretion in hypertensive subjects by potassium intake is less than that in normotensive subjects; and the increase in white subjects is higher than that in black subjects (79). The cells of connecting tubules, which synthesize and secrete urinary kallikrein, seem to participate in the process of potassium secretion. A recent electron microscopic study (80) revealed that a high-potassium diet produces hypertrophy and hyperplasia of the kallikrein-containing cells, including hypertrophy of the components of both the Golgi complex and rough endoplasmic reticulum and a large number of secretory-like vesicles containing kallikrein. The results suggest that a high-potassium diet increased the synthesis and secretion of kallikrein. It is well-known that aldosterone is synthesized and released from the glomerulosa cells of the adrenals. The glomerulosa cell is sensitive to changes in external potassium concentration and an infusion of 10 mEq of potassium over 30 min produces no measurable change in the serum potassium level of humans, but does increase plasma aldosterone levels by 25% (81). The transduction mechanism used by potassium is depolarization of the membrane with opening of the voltage-dependent calcium channels, and it is different from that used by angiotensin II, which is receptor-mediated (82).

Intravenous infusion of vasopressin (antidiuretic hormone) was reported to stimulate both the release of urinary kallikrein and the intrarenal formation of kinin in the dog and rat (83).

We have found that oxytocin is a renal kallikrein releaser (84). Intravenous infusion of oxytocin in Sprague-Dawley (SD) strain rats accelerated kallikrein secretion in accordance with increases in urine volume and urinary sodium excretion (see section V, 2 a). It remains questionable whether endogenous oxytocin plays the same role, but endogenous accelerators of renal kallikrein release
must be present, since there must be biochemical links between sodium accumulation and the acceleration of the secretion of renal kallikrein.

b. Kallikrein inhibitors

Apart from inhibitors in the plasma such as α₁-antitrypsin inhibitor, which inhibit urinary kallikrein (85), a tissue kallikrein inhibitor, kallistatin, has been isolated and purified, and its cDNA sequence has now been clarified (86-88). Kallistatin inhibits human tissue kallikrein activity toward either kininogen or a tripeptide substrate and belongs to the serpin superfamily, including protein C inhibitor, α₁-antitrypsin, and α₁-antichymotrypsin. The cDNA sequence of the kallikrein binding protein shares 68.8% identity with human α₁-antichymotrypsin. This protein is expressed at high levels in the liver and at low levels in the lung, salivary gland and kidney (86). In the kidney, the mRNA of this protein can be detected most abundantly in the inner medullary collecting duct, with small amounts in the outer medullary collecting duct, proximal convoluted tubules and the glomerulus (89). No signals are found in the connecting tubules or the cortical collecting duct (89). It is interesting that kallistatin is localized in tubules distal to the secretion site of renal kallikrein, the connecting tubules, and that it works immediately after kinin is generated and bound to the receptors in the collecting duct. SHR show lower levels of kallistatin in the serum, lung, heart, sali-
vary glands and kidney than Wistar Kyoto rats (86).

c. Kininogens

Kininogen was detected in human urine (90, 91). By the use of antibody against the heavy (H) chains of both kininogens, LMW kininogen was isolated, and the H chain antigen was localized in the kidney, where it was diffusely distributed in cells of the distal tubules and in the cortical and medullary collecting ducts. No intact HMW kininogen was found in the kidney or urine (92). Immunoreactive kininogen was localized in the principal cells of the collecting ducts and is restricted to the luminal portion of the principal cells (93). Immunoreactive tissue kallikrein was detected in the cells of the connecting tubules, the segment of the nephron preceding the cortical collecting ducts. Figure 6 shows the co-existence of tissue kallikrein and kininogen in the same transitional tubules, but in different cells (93, 94). The close relationship between cells that contain tissue kallikrein and kininogen suggests that kinins could be generated in the lumen of the collecting tubules. The mRNA of LMW kininogen is expressed in the renal cortex and medulla (95), suggesting the biosynthesis of LMW kininogen in the distal tubule.

A study with the mutant kininogen-deficient Brown Norway-Katholiek (mutant BN-Ka) rats indicated that intravenous infusion of partially purified rat LMW kininogen increased kinin excretion in ureter urine, whereas that of HMW kininogen caused a slight increase in kinin in the urine (96), indicating that the kidney secretes LMW kininogen and urinary kallikrein releases urinary kinin mainly from LMW kininogen.

Antigen against HMW kininogen, which was taken as an indicator of kininogens, was immunohistochemically localized at the distal tubules, but the intensity of the antigen immunostaining was similar in both normal BN-Kitasato (BN-Ki) rats and kininogen-deficient BN-Ka rats. HMW kininogen antigen in mutant BN-Ka rats was almost the same as that in normal BN-Ki rats. HMW kininogen was predominantly found in the microsomal fractions of kidney homogenates. The uptake of radiolabeled HMW kininogen by the tubular cells after incubation was only 0.6%. The mRNA of HMW kininogen, visualized by polymerase chain reaction amplification had almost the same intensity in the two strains of rats (97). As far as HMW kininogen is taken as an indicator, the results suggest that kininogens may be locally synthesized in the connecting tubules even in the kidney of mutant BN-Ka rats. The mutant BK-Ka rats can synthesize kininogens in the liver, but cannot release them from this organ into the blood stream (see below). In the same way, the connecting tubular cells can synthesize kininogens, but may not release them into the lumen. This could be accounted for by the fact that kinin cannot be detected in the urine of mutant kininogen-deficient BN-Ka rats.

![Diagram of the immunocytochemical localization of kallikrein and kininogen in the human nephron (a) and a schematic representation of the intermingled CNT cells and principal cells at the junction between CNT and CCD (b). AA, afferent arteriole; G, glomerulus; EA, efferent arteriole; PT, proximal tubule; LH, loop of Henle; MD, macula densa; DCT, distal convoluted tubule; CCD, cortical collecting duct; CNT, connecting tubule. (quoted from Ref. 93 with permission)]
d. Kininases

Kininases, which inactivate plasma kinins, are distributed in two major parts of the nephron: in the proximal tubules and medullary collecting duct. The micropuncture technique revealed that almost all of the $[^3H]BK$ injected into the proximal tubules is destroyed in the proximal tubules (98). It was reported that kininase II is concentrated in the proximal tubules along the brush border membrane of the cells or the S3 proximal tubule segments of the proximal tubules (99–101). Determination of kininase activity in the individual segments by the microdissection technique indicated that kininase activity is not only found in the proximal tubules, but is also present in the medullary collecting duct (100). Neutral endopeptidase (NEP) is also present in the outer surface of the brush border plasma membrane of the proximal tubules, and to a lesser extent, in the vesicular organelles both in the apical cytoplasm and on the basal infoldings of the proximal tubule cells (102). Stop-flow experiments suggest that NEP is localized in the distal tubule site (103, 104), but other researchers reported that no immunolabeling of this enzyme is observed in the distal portion of the nephron (102). Biochemical analysis of rat urine indicates that NEP accounts for 68% of the total kininase activity in rat urine, while kininase II and kininase I account for 23% and 9%, respectively (105). Urinary NEP accounts for more than half of the renal kininases in humans (106).

A kinin-hydrolyzing enzyme, which does not respond to inhibitors of the kininase I and II family of enzymes, is localized along the cortical and medullary collecting tubules of the rabbit (100). A new kininase I type (or carboxypeptidase type) enzyme was purified from human urine and kidney tissue. It differs from circulating kininase I in size, inhibitory profile and immunogenic specificity (107).

Our study (108) on the degradation pathways of BK reveals that the pathway in rat urine is completely different from that in rat or human plasma (109), as shown in Fig. 7. In the latter, the major metabolite of BK during incubation with plasma in vitro is BK-(1–5) or Arg-Pro-Gly-Phe (109), whereas during incubation of BK with rat urine, BK-(1–6) is the major metabolite and BK-(1–5) was not detected (108). Further analysis of kininases in rat urine revealed that the main kininases are NEP and carboxypeptidase Y-like exopeptidase (110), the latter of which was originally found in yeast. ACE inhibitors such as captopril or lisinopril scarcely inhibited the activity of these enzymes, but ebelactone B, isolated from the culture medium of actinomycetes, inhibits the activity of carboxypeptidase Y-like kininase in rat urine, without inhibiting plasma kininases. Treatment of anesthetized SD strain rats with ebelactone B during the infusion of physiological saline (6 ml/kg/hr) markedly increases the kinin levels in the urine and exerts diuretic and natriuretic actions (111).

**Fig. 7.** Pathways of bradykinin degradation by rat urine and rat plasma. Bradykinin-(1–n) indicates bradykinin degradation products with n amino acids from the N terminal.
e. Kinin receptors

The \[^{[H]}\text{BK}\] binding capacities along the nephron of the rabbit are maximal at the cortical collecting duct and outer medullary collecting duct and marginal at glomeruli, distal straight tubules and distal tubules (112, 113). BK inhibits net sodium absorption without affecting net potassium transport or the transepithelial potential difference (114). BK inhibits net chloride absorption, but does not affect the transepithelial voltage or the bicarbonate flux (115). Two types of BK receptors, B1 and B2, have been recognized (116). The B2-receptor seems to be present in the nephron, since the natriuretic and diuretic effects of BK are antagonized by the selective B2-antagonist Hoe 140 (111). Using chemically crosslinked conjugates of bovine serum albumin and the B2-agonist of BK or the potent B2-antagonist Hoe 140, the receptor has been found in straight portions of the proximal tubules, distal straight tubules, connecting tubules and collecting ducts of the rat kidney (117). The B2-receptors are present in the luminal membranes, in the basal infoldings of the tubule cells and in the smooth muscle cells of the cortical radial artery and of afferent arterioles. The B2-receptors are co-localized with kallikrein and kininogens in the connecting tubules and collecting duct cell layers, respectively.

Short summary: Kinins generated by the plasma kallikrein-kinin system may not have an important role in hypertension. In contrast, renal distal nephrons possess a full set of kallikrein-kinin system components and thus act as the “renal” kallikrein-kinin system, which works independently from other kallikrein-kinin systems for excretion of sodium and water.

II. Renal kallikrein-kinin system and hypertension

1. Hypertensive patients

In 1934, Elliot and Nuzum had already noticed that hypertensive patients without clinically apparent renal disease have significantly lower levels of urinary kallikrein than normotensive subjects (118). This abnormality in human hypertension was not confirmed until 1971.

Margolius et al. (71) reported lower levels of urinary kallikrein in patients with essential hypertension than in a control population, normal levels in patients with renal artery stenosis, and raised levels in patients with phaeochromocytoma and primary aldosteronism (Fig. 4). Since that time, multiple studies have been carried out in various individuals with hypertension and animal models of hypertension, showing similar findings of lowered kallikrein excretion in hypertension (119–129).

However, there were indications that variables such as race and renal function must be considered (130). Kallikrein excretion in white hypertensive men was lower than that in white normotensives during normal sodium intake, but was not different from that in black hypertensives and black control subjects under the same conditions. The kallikrein levels in the urine of normotensive black subjects are significantly lower than those in normal white subjects. All groups have greater urinary kallikrein activity on a low-sodium diet vs a unrestricted sodium intake, but the increase in black hypertensives is small. Plasma renin activity shows similar increments after sodium restriction in all groups. Similar results on reduced excretion of urinary kallikrein in black subjects were obtained (131).

Patients with malignant essential hypertension excrete less urinary kallikrein than those with non-malignant essential hypertension and normotensive control subjects (132). Some studies have reported that white patients with uncomplicated essential hypertension show normal kallikrein excretion rates with normal plasma renin activity and aldosterone (133). Only hypertensives over 40 years old excrete a significantly lower excretion of urinary kallikrein (134). Another report states that 20% of the hypertensive patients show low kallikrein excretion (135). This low excretion rate may be accompanied with low plasma renin activity (136), but there was no significant difference between the urinary kallikrein excretion of patients with low renin essential hypertension and those with normal renin essential hypertension in either black or white patients (137). Thus, the lower kallikrein excretion in essential hypertension is still controversial. Japanese patients with low-renin hypertension show significant reductions in both active urinary kallikrein and kinin excretion, together with increased levels of a kallikrein inhibitory material and kininase in urine and with reduced level of kininogen (138).

Kallikrein excretion was decreased in hypertensive patients with mild renal insufficiency (137). Although no significant difference in the urinary kallikrein excretion of patients with low-renin essential hypertension was found, hypertensive patients with mild renal insufficiency showed reduced urinary kallikrein excretion (137). Patients with reduced glomerular filtration rates showed markedly decreased urinary kallikrein excretion, like those with hypertension (139). Renal parenchymal diseases with hypertension, such as chronic glomerulonephritis, are associated with diminished urinary kallikrein activity (137). Rats with renovascular hypertension have decreased kallikrein levels both in renal tissue and in urine (120, 140). In two kidney-one clip Goldblatt hypertensive rats, the urinary kallikrein level was low in the urine from the stenotic kidney, whereas that of the contralateral kidney was normal (141). In Dahl salt-sensitive rats fed a normal sodium diet (0.45% NaCl), the urinary kallikrein level...
determined by the kinin generating activity, is lower than the level determined by direct radioimmunoassay for the enzymic protein (142). The level of urinary protein is higher in these rats (142). The lower level of the kallikrein may be due to inhibitors leaking from the plasma. The reduced levels of kallikrein in hypertension should be distinguished from those due to impaired renal function.

However, recent studies suggest a strong influence of urinary kallikrein excretion on the salt-sensitivity of blood pressure in normotensive patients (143). In a randomized cross-over double-blind study, the urinary excretion of active kallikrein was significantly lower in salt-sensitive hypertensives than in salt-resistant hypertensive patients, and it showed an inverse correlation with plasma atrial natriuretic peptide (ANP) levels (144). Thus, at least some of the hypertensive patients excrete lower levels of kallikrein without reduced renal function.

2. Animal models of hypertension

Reduced excretion of urinary kallikrein was also reported in genetically hypertensive rat models (140, 141, 145, 146), in rats made hypertensive by deoxycorticosterone plus 1% salt, and in rats receiving deoxycorticosterone alone (140). Rats of the genetically hypertensive New Zealand strain excreted reduced levels of urinary kallikrein (145). The urinary excretion of kallikrein by hypertensive Fawn-Hooded (FH/Wjd) male and female rats was less than that of Wistar rats (and male < female in urinary kallikrein excretion) from 1.5 months before the hypertension developed at the ages of 2 (male) and 4.5 (female) months (146). FH male rats excreted more sodium and urine than all other groups. Only FH male rats developed proteinuria, but neither an inhibitor of urinary kallikrein nor increased degradation of this enzyme in the urine was found in FH rats.

In Okamoto-Aoki SHR, kallikrein excretion was subnormal (140, 147–150). A time course study (148) revealed that urinary excretion of active and total kallikrein was significantly lower in the SHR on a normal sodium diet from 4 through 15 weeks of age. The average values of active and total kallikrein activity in the SHR were 69.5% and 67.4%, respectively, of the values in age-matched Wistar-Kyoto rats (WKY) throughout the development of hypertension, even after reaching a plateau of systolic

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**Fig. 8.** Changes in the activities of urinary kallikrein (A), and prokallikrein (B), systolic blood pressure (C), and plasma renin activity (D) of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY). Values in SHR were compared with those in WKY. *P < 0.05, **P < 0.01. (quoted from Ref. 151 with permission).
blood pressure at 10–11 weeks. SHR exhibited a lower urinary excretion of sodium and water than WKY, together with a higher cumulative sodium balance at all ages studied and a higher cumulative water balance only at 7 and 8 weeks of age. In response to an acute decrease in renal perfusion pressure, the slopes of the regression lines correlating urinary kallikrein to systolic arterial pressure to urinary excretion and to the cumulative balance of sodium and water were always significantly less in SHR than in WKY, indicating reduced excretion of urinary kallikrein (148).

The reduced urinary kallikrein excretion in SHR was also confirmed during the development of hypertension (151), but the difference of the urinary kallikrein level between SHR and WKY disappeared when the systolic pressure reached a plateau at the age of 10 weeks (Fig. 8). Thus, this result does not agree with the report that lower excretion of urinary kallikrein persists after the blood pressure has reached a plateau (148). The reason for this discrepancy is not clear. The reduced excretion of not only sodium, but also potassium and creatinine with an increased serum creatinine level from the age of 4 weeks (weanlings) (151) may suggest renal insufficiency. Abnormalities in glomerular function in rats developing spontaneous hypertension were reported in clearance and micropuncture studies in 6-week-old SHR (152). Nevertheless, the fact that the inhibition of urinary kallikrein in SHR with aprotinin, a polyvalent serine esterase inhibitor, increased the systolic blood pressure during the development of hypertension (for 3 days from the age of 7 weeks) (151) indicates that the urinary kallikrein excreted still accelerates sodium excretion and suppresses increases in the systolic blood pressure during the development of hypertension in SHR, even if the urinary kallikrein level is reduced at the developmental stage of hypertension. However, the kallikrein activity reduced by impaired renal function caused by the continuing hypertension should be carefully distinguished from the original reduction of kallikrein activity.

The reduced urinary kallikrein level (68% to 66%) of SHR is not due to a defect in synthesis by the renal cortex at birth, but to a defect in prokallikrein activation, since the total kallikrein is not reduced in the renal cortex of newborn SHR when compared with that in WKY, while active urinary kallikrein is reduced (149). During the development of hypertension from 4 to 12 weeks of age, the renal content of both active kallikrein and total kallikrein relative to the renal cortex weight is reduced, although the active kallikrein content per g of cortex weight is increased at 4, 8 and 12 weeks of age (149). When their diet was switched from a 0.4% to a 0.0064% sodium chloride diet, normotensive Dahl salt-sensitive rats failed to reach the maximum in kallikrein activity or total kallikrein (150). The enzymatic activity of renal tissue kallikrein per mg of renal tissue protein increased from 4 to 52 weeks in SHR, but fell at 78 weeks as a result of tubular atrophy and fibrosis in advanced hypertension; and this was also observed in human biopsy specimens (153). The ratio of active kallikrein to prokallikrein in urine has been reported to be near unity (149) and 50% (154). As renal kallikrein is a mineralocorticoid-regulated protein, renal kallikrein messenger RNA levels were studied, but no differences were found between adrenalectomized rats and those treated for 5–14 days with 9α-fludrocortisone, corticosterone or dexamethasone, or between SHR and their appropriate controls, so that the changes in renal kallikrein activity and immunoreactivity after long-term mineralocorticoid administration in adrenalectomized rats and in genetically hypertensive rats may reflect modulation at the post-transcriptional level (155). These results suggest that genetically hypertensive rats may have a disposition towards reduced secretion of urinary kallikrein.

Adducin is a cytoskeletal protein that interacts with other membrane-skeleton proteins that affect ion transport across the cell membrane. In the Milan hypertensive strain of rats, which show fast ion transport across the cell membrane (156–158), the cDNA of adducin sequences shows a one-point mutation in each of the two genes coding for the α- and β-subunits of adducin (159). A case-control study to test the association between the α-adducin locus and hypertension revealed that a polymorphism within the α-adducin gene may affect the blood pressure in humans (160). These results, taken together, cannot exclude the possibility that the reduced excretion of urinary kallikrein in congenitally hypertensive rats may be attributable to a disorder in the excretion of active kallikrein due to an insufficiency of the cytoskeletal protein for secretion.

3. Genetic background

The separation of Okamoto-Aoki genetically hypertensive rats from WKY indicates the importance of genetic factors in the development of hypertension. Similar hypertensive rat strains have also been reported (140, 141, 145, 146). The most convincing evidence of the pivotal role of the kidney was provided by the experiments on cross-transplantation of kidneys between normotensive and spontaneously hypertensive strains (161, 162). Normotensive recipient rats, which received SHR donor kidneys even in the prehypertensive stage (5–6 weeks of age), had significantly higher values of blood pressure and serum urea (161). When the F1 hybrids between SHR and Wistar rats received a kidney from SHR, they showed higher blood pressure than Wistar rats with low renin activity both in the plasma and the kidney (162). Similar
evidence was provided by rats of the Dahl salt-sensitive and salt-resistant strains, which showed opposite genetic propensities for hypertension (163). Using a parabiotic technique, the authors predicted a factor associated with the salt excretion mechanism, which is specific for the hypertension-prone strain as well as a factor transmittable via the parabiotic junction (renin-angiotensin system) (164). In interstrain renal transplants between the salt-sensitive and salt-resistant strains of rats (165), kidneys from the hypertension-prone rats exerted a prohypertensive effect, while those from hypertension-resistant rats generally had an anti-hypertensive effect. These effects on blood pressure were most clear-cut in rats maintained on a low-sodium diet (0.3% NaCl), indicating that the kidney of the donor may be a genetic determinant. The renal involvement in the development of hypertension is suggested by the results of electrolyte-balance studies demonstrating a period of relative sodium and water retention in SHR. The dietary sodium restriction retards the development of hypertension in SHR, but does not prevent the hypertension (166). In rats of the Milan hypertensive strain younger than 9 weeks of age, the sodium retention observed is due to a significantly lower urinary excretion of dietary sodium (167).

It is quite feasible that the mechanism of development of hypertension may spring from abnormalities in the renin gene. In an F2 population derived from crossing Dahl salt-sensitive rats and salt-resistant rats, a restriction fragment length polymorphism (RFLP) in the renin gene cosegregated with blood pressure. One dose of the salt-sensitive rat renin allele was associated with an increment in blood pressure of approximately 10 mmHg, and two doses of this allele increased blood pressure approximately 20 mmHg (168). In Southern blotting using cDNA and an oligonucleotide probe of the SHR renin gene, a “deletion” of around 650 base pairs was found in the first intron (intron A) of the SHR gene, in comparison with the WKY gene (169). However, another study (170), which examined the inheritance of a DNA RFLP in the renin gene in an F2 population derived from inbred SHR and inbred normotensive Lewis rats, indicated that the blood pressure in rats that inherited a single SHR renin allele (1.7-kb band) was significantly higher than that in rats that inherited only the Lewis renin allele (2.7-kb band). However, Dahl salt-sensitive rats exhibit a 1.7-kb band, which is also seen in Lewis rats, and Dahl salt-resistant rats exhibit a 2.7-kb band, which is also carried by SHR. Thus, although a structural alteration in the renin gene may be present in congenitally hypertensive rats, considerable difficulties remain before the hypertension in these animals can be explained by the altered renin gene.

Although the inherited susceptibility or resistance to the effect of salt is polygenetic, the kidney appears to play a primary role in the determination of blood pressure (165, 171, 172). This relation may be presented on the gene basis (173). Molecular evidence of an association between a sequence alteration in the kallikrein gene family and the transmission of increased blood pressure has been presented. In recombinant inbred (RI) strains derived from SHR and normotensive BN rats, the RI strains that inherited RFLP of kallikrein from the SHR progenitor strains (6.4-kb fragment) show significantly greater median systolic, diastolic and mean arterial pressures than the RI strains that inherited the kallikrein RFLP from the BN progenitor strains (173).

Segregation analysis on a large number of Utah pedigrees, covering 1.2 million subjects (approximately 30% of the current Utah adult population) as well as 140,000 Utah death certificates over a 20-year period, was carried out to find the genetic and environmental determinants of lipid abnormalities and coronary arterial disease (1).

A large-scale epidemiologic study provides some information about possible longer-term relationships between urinary kallikrein and blood pressure (174, 175). In a population of more than 700 healthy children aged 2–14 years, a familial aggregation of high blood pressure was found in children studied for 15 years. Urinary kallikrein, which was also aggregated in families, was lower in black children than white children and was inversely related to blood pressure. Similar significant inverse relationships between urinary kallikrein or creatinine concentration and blood pressure were found in white and black children, and they were relatively stable over an eight-year period of observation (175).

A study with 405 normotensive adults and 391 youths in 57 Utah pedigrees provided evidence that total urinary kallikrein excretion was highly familial, with 51% of the total variance attributable to a dominant allele for high total urinary kallikrein excretion and 27% attributable to the combined effects of polygenes and shared family environment (11). An estimated 28% of the population has one or two copies of the dominant allele for high total urinary kallikrein excretion. About 83% of the population could be assigned to one of the two genotypic populations. Individuals with the high total urinary kallikrein excretion genotype were significantly less likely to have one or two hypertensive parents (11). Using the same analysis on large Utah pedigrees, significant statistical urinary potassium interaction with the inferred major gene for kallikrein was found (12): The heterozygote kallikrein group (with a frequency of 50%) shows a significant association between urinary kallikrein and urinary potassium, whereas there was no association with potassium in the low homozygotes. The model predicted that an increase in urinary potassium excretion in these
Pedigrees would be associated with high kallikrein levels in the heterozygotes similar to the high levels in the homozygotes, and that a decrease in urinary potassium excretion in heterozygous individuals would be associated with kallikrein levels similar to the levels in homozygous individuals with low kallikrein (12). Because, in the steady state, urinary potassium represents dietary potassium intake, this study suggests that an increase in dietary potassium intake in 50% of these pedigree members, estimated to be heterozygous at the kallikrein locus, would be associated with an increase in an underlying genetically determined low kallikrein level (12). Urinary potassium, pH and systolic blood pressure differences explained 34% of the differences in kallikrein levels between monozygous twins (176), suggesting an additional unmeasured environmental variable that is associated with decreased kallikrein excretion and elevated blood pressure.

On the basis of these observations, Williams (1) proposed the following hypothesis (Fig. 9): subjects can be divided into three kallikrein genotypes; approximately half will be heterozygous for this single-gene trait. In this population with the heterozygous genotype, low potassium intake would have a high susceptibility to hypertension, whereas high potassium intake would reduce the risk of hypertension. Kallikrein levels in approximately 30% of the population are low in "low homozygotes", who have a high risk of hypertension. Approximately 20% of the population are, according to segregation analysis, "high homozygotes", who are at a low risk of hypertension regardless of potassium intake (1).

The hypertensive effect of dietary potassium intake is controversial, but a randomized, cross-over, double-blind study conducted for four days on 22 patients of ≥60 years old revealed a decrease in systemic blood pressure during potassium chloride ingestion (120 mmol/day) (177). As more sodium, potassium and aldosterone were excreted during the daytime, while urinary kallikrein was excreted at a fixed rate throughout both day and night (178), a long-term study may be necessary. Nevertheless, it is highly likely that ordinary essential hypertension occurs in people who have susceptibility genes at both angiotensinogen (5) and kallikrein loci, as long as they consume a high-sodium, low-potassium diet (1).

Short summary: Low excretion of urinary kallikrein has been reported in hypertensive patients and congenitally hypertensive animals, but the effects of the low kallikrein excretion have not been clearly identified. Nevertheless, the extensive genetic and environmental studies on large Utah pedigrees suggest that the development of hypertension is strongly related to the combination of a lower kallikrein excretion genotype and potassium intake.

III. Congenital deficiency in the kallikrein-kinin system

Despite a large body of references suggesting the importance of the role of the urinary kallikrein-kinin system in the development of hypertension, direct and definitive evidence providing the missing link between reduced excretion of urinary kallikrein and the development of hypertension are lacking. The main reason for this may have been that it was impossible to eliminate the kinin components from living animals. However, this has been successfully achieved using mutant rats devoid of kininogens, the precursors of kinins, in the plasma.

1. Mutant Brown Norway-Katholiekg rats

Mutant rats of the Brown Norway (BN) strain (Rattus norvegicus, BN/fMai) were discovered at the Katholieke University of Leuven, Belgium, and were reported to be devoid of kallikrein-like activity and have a low level of kininogen in plasma (179–181). This was confirmed by another group (182). This BN strain of rats show a prolonged kaolin-activated partial thromboplastin time due to lack of HMW kininogen and low level of plasma prekallikrein (183). They were designated as BN-Katholiekg (BN-Ka) rats (182). Further studies revealed that both HMW and LMW kininogens were almost entirely absent from the plasma (184, 185) (Fig. 10), and they are practi-
cally incapable of excreting kinin in their urine (185, 186) (Fig. 10). Normal rats of the same strain were kept at the Kitasato University animal facilities and were designated as BN-Kitasato (BN-Ki) rats (182). They show the same levels of kininogens as rats of other strains, such as the SD strain (185). The mutant BN-Ka rats are capable of producing kininogens in the liver, but cannot release them into the blood stream, because of a point mutation of Ala163 to threonine in the structure of the kininogens (187). The HMW, LMW and prekallikrein mRNA are present in the liver of BN-Ka rats with a similar size and abundance, compared with BN/Orl rats (188). The roles of the plasma kallikrein-kinin system in inflammation using these mutant BN-Ka rats (189) have been reviewed (190).

Congenital deficiency of kininogens in the plasma was also reported in humans (191–194). We reported (195, 196) the first case in Japan of kininogen-deficient twin sisters (Fujjwara trait), who are congenitally deficient in HMW and LMW kininogens in the plasma, have reduced levels of plasma prekallikrein, and show prolongation of

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**Fig. 10.** Kininogen levels in plasma (upper panel) and urinary kinin excretion (lower panel) in normal Brown Norway Kitasato (BN-Ki) rats and mutant BN-Kathiokiek (BN-Ka) rats. Values are means±SEM of four rats. BK eq, bradykinin equivalent; HMW, high molecular weight; LMW, low molecular weight.

(quoted from Ref. 185 with permission)

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**Fig. 11.** Changes in systolic blood pressure in normal Brown Norway-Kitasato (BN-Ki) rats and mutant BN-Katholiek (BN-Ka) rats given NaCl-loaded diets. Both strains of rats were fed NaCl diets from 2% to 8% from the age of 7 weeks for two weeks (Panel A) and a 2% NaCl diet between the ages of 7 and 11 weeks (Panel B). Values are means ± SEM of 7–12 rats. Values in BN-Ka rats were compared with those in BN-Ki rats of the same age. **P<0.01, ***P<0.001.

(quoted from Ref. 199 with permission)
the activated partial thromboplastin time, since HMW kininogen and plasma kallikrein are essential in the activation of coagulation factor XII. However, the sisters displayed no apparent clinical symptoms and underwent appendectomy without excessive bleeding (195). Susceptibility to salt and hypertension has not been studied. A similar kininogen-deficient family has also been discovered in Japan (197).

Like kininogen-deficient humans, mutant kininogen-deficient BN-Ka rats have no apparent symptoms. The change of the systemic blood pressure during growth in mutant BN-Ka rats is the same as in normal BN-Ki rats, when they take 0.3% NaCl in the diet and drink distilled water (185) (see Fig. 15). The dose-response curve of angiotensin II injected intravenously into mutant BN-Ka rats is not different from that in normal BN-Ki rats, suggesting that the arteriolar smooth muscle in the former animals is no more sensitive to angiotensin II than that of the latter (198). Breeding of mutant BN-Ka rats between sisters and brothers is not easy, because the breeding rate is low. Nevertheless, the following experimental results clearly indicate that mutant BN-Ka rats are very sensitive to ingested salt, which causes sodium accumulation and consequent hypertension. Furthermore, sodium accumulation is also readily induced by aldosterone released by a non-pressor dose of angiotensin II.

2. Hypertension induced by low salt loading in mutant BN-Ka rats

Feeding normal BN-Ki rats with a diet containing increasing concentrations of NaCl caused increases in systolic blood pressure, measured by the tail cuff method, when the dietary concentration of NaCl exceeded 4% (199) (Fig. 11A), whereas kininogen-deficient BN-Ka rats

![Fig. 12. Bar graphs show changes in water intake (A), and urine volume (B), and urinary excretion of sodium (C), potassium (D), and creatinine (E) in normal Brown Norway Kitasato (BN-Ki) rats and mutant BN-Katholiek (BN-Ka) rats. Values are means ±SEM of n rats. After measurement at 7 weeks of age, the diet was changed from low NaCl (0.3%) to 2% NaCl. Values in BN-Ka rats were compared with those in BN-Ki rats at the same age. *P < 0.05. (quoted from Ref. 199 with permission)
showed an increase in systolic blood pressure after receiving only 2% of NaCl in their diets. Figure 11B shows the changes in the systolic blood pressure of rats of both strains fed with a 2% NaCl diet for four weeks. In the mutant BN-Ka rats, the systolic blood pressure increased up to 167 ± 4 mmHg, whereas that of normal BN-Ki rats did not change during the four-week period. During the period of feeding with the 2% NaCl diet, both strains of rats showed increases in water intake and urine volume, but mutant BN-Ka rats ingested more water and excreted less urine than the normal BN-Ki rats (Fig. 12) (199), so that the tentatively calculated difference (water intake minus urine volume) was much larger in the former than in the latter, which was constant during the four-week period. Urinary excretion of sodium also increased, but mutant BN-Ka rats excreted less than the normal BN-Ki rats (Fig. 12). Urinary excretions of potassium and creatinine were not different between normal BN-Ki rats and mutant BN-Ka rats. Despite the reduced excretion of sodium and water in mutant BN-Ka rats, their serum sodium level increased slightly, whereas that of normal BN-Ki rats was constant. Interestingly, the sodium levels in the erythrocytes during the 2% sodium loading were increased significantly in the mutant BN-Ka rats, but remained constant in the normal BN-Ki rats. Plasma renin activity was reduced and then tended to increase, but there was no difference between the two strains.

A 7-day subcutaneous infusion of LMW kininogen by a mini-osmotic pump, implanted subcutaneously in the back, performed from day 8 in kininogen-deficient BN-Ka rats fed a 2% NaCl diet lowered the systolic blood pressure to the control level, together with increases in urinary kinin, and sodium excretion and in urine volume. In contrast, subcutaneous infusion of the BK B2-antagonist Hoe 140 into normal BN-Ki rats fed a 2% NaCl diet resulted in an increase in systolic blood pressure to 166 ± 23 mmHg, which was significantly higher than the systolic blood pressure of normal BN-Ki rats receiving the physiological saline vehicle. The increase in systolic blood pressure in normal BN-Ki rats was accompanied by reduced excretion of urinary sodium and reduced urine volume.

These results clearly indicate that mutant BN-Ka rats are extremely sensitive to ingested salt and shows a direct relationship between kininogen deficiency or lack of kinin generation, sodium excretion and increase in systolic blood pressure.

As shown in Fig. 11A, dietary sodium concentrations of over 4% increase systemic blood pressure even in normal BN-Ki rats. Usually, 7–9% of the sodium concentrations is used for the induction of experimental hypertension in normotensive rats. However, it should be kept in mind that excretion of active urinary kallikrein, not urinary prokallikrein, is also reduced by the intake of more than 4% of sodium in the diet (Fig. 13). Accordingly, hypertension experiments with high sodium concentrations in the diet may have been carried out while the urinary kallikrein levels were reduced without the researchers’ knowledge.

3. Hypertension induced by a non-pressor dose of angiotensin II in mutant BN-Ka rats

Even during feeding with a 0.3% sodium diet, excess aldosterone release, e.g., by a low dose of angiotensin II, induces hypertension in mutant BN-Ka rats. Subcutaneous infusion of a non-pressor dose (20 μg/day/rat) of angiotensin II in normal BN-Ki rats with a mini-osmotic pump for two weeks did not change the systolic blood pressure (Fig. 14A), but the same treatment in mutant BN-Ka rats caused hypertension (180 ± 8 mmHg), sug-
gesting that hypertension may not be due to direct vasoconstriction by this substance, but to other factors (198). The heart rate was also increased markedly (Fig. 14B). The serum sodium level was significantly increased, and the hematocrit decreased, in deficient BN-Ka rats. The sodium level in the erythrocytes rose gradually during

![Graph A](image.png)  
Fig. 14. Changes in systolic blood pressure (A), heart rate (B), and sodium concentration in erythrocytes (RBC[Na]) (C) in normal Brown Norway-Kitasato (BN-Ki) rats and mutant Brown Norway Katholiek (BN-Ka) rats during infusion of low-dose angiotensin II (Ang II). Values show mean±SEM of the numbers (n) of rats. After blood pressure measurement at 7 weeks of age, Ang II (20 μg/d per rat SC) was infused for 2 weeks. Spironolactone (50 mg/d per rat) was given to Ang II-treated BN-Ka rats for 7 days. Values in BN-Ka rats were compared with those in BN-Ki rats at the same age; *P<0.05, **P<0.01, ***P<0.001. Values in BN-Ka rats with spironolactone were compared with those in BN-Ka rats receiving only Ang II; #P<0.05. (quoted from Ref. 198 with permission).

![Graph B](image.png)  
![Graph C](image.png)  
Fig. 15. Graphs show inhibitory effects of continuous subcutaneous administration of kininogen on increases of systolic blood pressure (A), heart rate (B), and erythrocyte sodium concentration (RBC[Na]) (C) in mutant Brown Norway Katholiek (BN-Ka) rats given low-dose angiotensin II (Ang II). From 7 weeks of age, Ang II (20 μg/d per rat SC) was infused for 2 weeks. After blood pressure determination at 8 weeks of age, subcutaneous infusion of low-molecular-weight kininogen was started. Values show mean±SEM of the numbers (n) of rats and were compared between the group (closed circles, hatched column) and a vehicle control group (open circles, open column). *P<0.05. (quoted from Ref. 198 with permission)
subcutaneous infusion of angiotensin II in mutant BN-Ka rats (Fig. 14C), and that in the cerebrospinal fluid was also markedly increased, suggesting that sodium was accumulated in the body fluid and the cells.

Simultaneous subcutaneous infusion of spironolactone, an aldosterone antagonist, with angiotensin II in mutant BN-Ka rats in the second week of the angiotensin infusion period reduced the high systolic blood pressure to the level seen in the normal BN-Ki rats (Fig. 14A). Simultaneously, the increases in heart rate (Fig. 14B) and in the sodium levels in the erythrocytes (Fig. 14C) and the cerebrospinal fluid returned to the normal BN-Ki rat levels during the spironolactone treatment, indicating that the aldosterone released by angiotensin infusion had induced both the hypertension and the increase in these parameters. Urinary secretion of aldosterone was increased during the angiotensin infusion, but there was no difference between the two strains of rats.

Because of the lack of plasma kininogens in the BN-Ka rats, supplementary LMW kininogen was infused for the second week of the angiotensin infusion period with a mini-osmotic pump. This supplementation markedly decreased the systolic blood pressure, heart rate and erythrocyte sodium levels (Fig. 15). By contrast, the subcutaneous injection of the BK B2-receptor antagonist Hoe 140 in normal BN-Ki rats during the second week of angiotensin infusion markedly increased the systolic blood pressure, heart rate and sodium levels in the erythrocytes. The possibility that the arterioles of deficient BN-Ka rats are essentially more sensitive to angiotensin II than those of normal BN-Ki rats was eliminated by the finding that in anesthetized rats of both strains, the dose-response curves of angiotensin II were not significantly different (198).

These results indicate that kininogen-deficient BN-Ka rats, incapable of generating kinin in the renal tubules, show lowered renal excretion of sodium and water and are readily susceptible to hypertension due to sodium accumulation, once either the diet is loaded with a low level of salt or aldosterone is released by angiotensin II. This was mimicked in the normal BN-Ki rats when they were treated with Hoe 140.

As mentioned before in animal models with hypertension (151), Okamoto-Aoki SHR excreted reduced levels of urinary active kallikrein and prokallikrein from the age of four weeks (the weaning period) to the time when the systemic blood pressure reached a plateau. The SHR also show higher plasma renin activity immediately after weaning (151) and the sodium level in erythrocytes was increased. These findings suggest that the induction of hypertension in kininogen-deficient BN-Ka rats by subcutaneous infusion of a non-pressor dose of angiotensin II is not specific to this strain, but may be a universal mechanism that also operates in genetically hypertensive rats such as Okamoto-Aoki SHR rats.

4. DOCA-salt hypertension in mutant BN-Ka rats
As in other strains of rats, a 1% sodium concentration in the drinking water with weekly subcutaneous injections of DOCA to uninephrectomized normal BN-Ki rats at 7 weeks of age caused a gradual increase in the systolic blood pressure, which reached a plateau (180 ± 10 mmHg) at 18 weeks of age (185) (Fig. 16). The same treatment in mutant BN-Ka rats increased the systolic blood pressure rapidly to 158 ± 6 mmHg within 2 weeks and then caused a further slight rise (Fig. 16). As Fig. 5 shows, the time courses of excretion of urinary kallikrein were the same in
both normal BN-Ki rats and mutant BN-Ka rats, so that urinary kallikrein activities peaked at 10 weeks and declined thereafter, following the changes in urinary sodium excretion and urine volume in normal BN-Ki rats. In contrast, mutant BN-Ka rats were unable to excrete sodium and water in the urine, and the systemic blood pressure increased very rapidly almost to its maximum within 3 weeks after the start of treatment. These time course studies indicate that the natriuresis due to the renal kallikrein-kinin system suppressed the systemic blood pressure rise in normal BN-Ki rats and that the decrease in the kallikrein activity to the preinjection level, even during the treatment, allows the development of hyper tension. These results clearly indicate that the urinary kallikrein-kinin system prevents the early increase of the systolic blood pressure in the DOCA-salt hypertension model by acceleration of sodium excretion and consequent prevention of the sodium accumulation that took place in the early phase.

5. Importance of sodium accumulation and increased vascular sensitivity in induction of hypertension

Low sodium loading or angiotensin II infusion in mutant BN-Ka rats increases the circulating blood volume with sodium retention, since the hematocrit value in deficient BN-Ka rats was decreased during angiotensin infusion, whereas no difference in hematocrit was observed after a similar infusion in normal BN-Ki rats (198). However, the following experiments (200) indicate that sodium accumulation plays a crucial role in the development of hypertension.

A large volume (6 ml/kg/hr) of 0.15 M or 0.3 M NaCl solution was infused intra-arterially for 4 days into conscious, unrestrained rats through an indwelling catheter. Infusion of normal BN-Ki rats with 0.15 or 0.3 M NaCl increased neither the mean arterial pressure (Fig. 17) nor the sodium levels in the serum, cerebrospinal fluid or erythrocytes. In contrast, infusion of the same volume of 0.3 M NaCl solution into mutant kininogen-deficient BN-Ka rats significantly increased the mean arterial pressure (Fig. 17), together with increase in the sodium levels in the serum, cerebrospinal fluid and erythrocytes, although infusion of 0.15 M NaCl solution did not change these parameters. The hematocrit values were not significantly changed in either strain by either infusion. Thus, sodium accumulation in the body is considered to be more important than the circulating blood volume in the development of hypertension.

Interestingly, after a 4-day infusion of 0.3 M NaCl solution to conscious mutant BN-Ka rats, the dose-response curve of the arteriolar response to angiotensin II shifted to the left, bringing about tenfold increases in the arteriolar responses to angiotensin II (Fig. 18). The arteriolar sensitivity to norepinephrine also increased 30-fold (200). The sensitivity of the arterioles of normal BN-Ki rats was not changed after infusion of either 0.15 or 0.3 M NaCl solution.

A similar increase in responsiveness was reported in hypertension models and hypertensive patients: Enhanced sympathetic control of the heart at baseline and in response to adrenergic stimulation was observed in a conscious canine perinephritic hypertension model during the development of hypertension (201, 202). This enhanced vascular responsiveness is attributed to the \( \alpha_1 \)-adrenergic receptor density in the membrane preparations from aortic tissue (203). Perfused segments of second-
order mesenteric resistance arteries from SHR show greater sensitivity to norepinephrine than those from WKY. This increase was due to depressed endothelium-dependent dilatation, since removal of the endothelium abolished the difference in sensitivity to norepinephrine between the two strains (204). In humans, normotensive subjects with positive family histories of hypertension are characterized by a higher sensitivity to angiotensin II in the systemic and renal circulation than in subjects with negative family histories of hypertension (205). In normotensive subjects with a positive family history of essential hypertension, the responsiveness of blood pressure to infused norepinephrine is exaggerated, and an increase in potassium intake may improve the norepinephrine hypersensitivity and simultaneously normalize the lower blood pressure (206). Furthermore, in borderline hypertensive patients and mild hypertensive patients during isometric exercise at 30% of maximum force for 3 min, the increase in blood pressure was mainly associated with an increase in peripheral resistance (207).

It has been reported (208) that bolus injections of increasing concentrations of NaCl into the cisterna magna of SD strain rats enhances the discharge of the sympathetic nerves in a concentration-dependent manner and increases the systemic blood pressure. Increased sympathetic drive is frequently observed in young hypertensive patients, particularly during the initial phases of hypertension (209). This increase may be caused by an accumulation of sodium in body, especially in the cerebrospinal fluid.

We may conclude from these results that failure of renal sodium excretion may result in sodium accumulation in the body, and that this sodium accumulation in erythrocytes or even in vascular smooth muscle causes an increased arteriolar response to vasoconstrictive substances such as angiotensin II and norepinephrine. The increased sodium concentrations in the cerebrospinal fluid enhanced the sympathetic discharge, further increasing the norepinephrine release. Thus, sustained increase in systolic blood pressure can be, at least partly, induced by increased sympathetic tone due to sodium accumulation.

Short summary: The discovery of mutant kininogen-deficient BN-Ka rats, which lack kinin generation in urine, may help to clarify the complicated mechanism of the development of hypertension. BN-Ka rats are very sensitive to ingested salt and to sodium retention by aldosterone released by a non-pressor dose of angiotensin II, and they are very susceptible to hypertension due to sodium accumulation in the cerebrospinal fluid and in the erythrocytes, and probably in the vascular smooth muscle. BN-Ka rats show an increased vasoconstrictive response even after a 4-day infusion of 0.3 M NaCl solution, resulting in hypertension. Thus, sodium accumulation in the body and lack of sodium excretion are crucial elements in the development of hypertension.
IV. Role of renal kallikrein-kinin system in sodium accumulation

The above mentioned studies on kininogen-deficient BN-Ka rats have clearly demonstrated that the renal kallikrein-kinin system, located along the nephron from the connecting tubule to the cortical collecting duct, plays a role in excretion of sodium. The failure of sodium excretion allows accumulation of sodium in the cells and cerebrospinal fluid. This is easy to accept, since BK is a potent natriuretic agent. However, the contribution of the kallikrein-kinin system to sodium excretion is probably minimal, when no excess sodium is taken up. The systemic blood pressure increases with age from the 4th week of age in normal BK-Ki rats and kininogen-deficient BN-Ka rats, but there is no difference in the systemic blood pressure between mutant BN-Ka rats and normal BN-Ki rats, when both are fed a 0.3% sodium diet (Fig. 16). In contrast, as mentioned above, 2% NaCl in the diet or the release of aldosterone by a non-pressor dose of angiotensin II accelerates sodium accumulation. Accordingly, the crucial role of the renal kallikrein-kinin system is to excrete the excess sodium. It is reported (210) that nearly 95% of the sodium filtered by the renal glomeruli is reabsorbed before reaching the cortical collecting duct. Furthermore, the tubuloglomerular feedback system may regulate the glomerular filtration rate depending upon the sodium concentrations in the macula densa of the tubules. Thus, if the amount of sodium exceeds the reabsorption ability of the tubules preceding the connecting tubules, sodium can reach the cortical collecting duct, where the BK B₂-receptors that can excrete the excess sodium, are distributed.

Therefore, we propose the hypothesis that the renal kallikrein-kinin system acts as a sort of floodgate for retained sodium. As shown in Fig. 19, normal BN-Ki rats or WKY fully open the floodgate of the renal kallikrein-kinin system. Once sodium begins to be accumulated in the body, either by excess salt loading or by aldosterone release by angiotensin II, the gate opens and the kinin generated in the cortical collecting duct inhibits sodium reabsorption and accelerates the excretion, thus preventing accumulation (22). In contrast, lack of kinin generation in the collecting duct, as in mutant kininogen-deficient BN-Ka rats, closes the floodgate; and a low dose (2%) of sodium or a release of aldosterone by angiotensin II may initiate the accumulation of sodium in the serum, cerebrospinal fluid and erythrocytes (22). The reduction

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Fig. 19. Role of the kallikrein-kinin system (KKS) in the kidney. BN-Ki, normal Brown Norway Kitasato rats; WKY, Wistar Kyoto rats; Deficient BN-Ka, kininogen-deficient Brown Norway Katholiek rats; SHR, spontaneously hypertensive rats; Ang, angiotensin II; Ald, aldosterone.

(quoted from Ref. 22 with permission)
of the gate size, as in SHR, together with the increase in renin release, may cause hypertension due to the sodium accumulation.

Renal kallikrein may be released into the basolateral side, and kinin released in the interstitial space may play some role in vasodilatation. Nevertheless, luminal kinin fulfills a much more important function. As mentioned above (108), the degradation pathways of BK in urine and plasma are quite different. Treatment of DOCA-salt hypertensive rats with a selective inhibitor of the carboxypeptidase Y-like endopeptidase ebelactone B suppressed the high blood pressure during the administration (211) (see section V, 1a). Poststatin, an inhibitor of both carboxypeptidase Y-like exopeptidase and NEP, also shows the same suppressive effect (212).

These results clearly indicate that the major site of action of BK, in relation to its regulation of systemic blood pressure, is not on the basolateral side of the collecting duct, but on the luminal side. Thus, kinin generated in the cortical collecting duct inhibits the reabsorption of sodium on the luminal side and accelerates the sodium excretion to prevent the development of hypertension.

Short summary: From the above-mentioned results, we hypothesize that the renal kallikrein-kinin system is a floodgate for accumulated sodium. If it is closed as in mutant BN-Ka rats, sodium is easily accumulated, and hypertension develops. When the gate size is restricted as in congenitally hypertensive rats, sodium again tends to be accumulated, and hypertension follows. Thus, the renal kallikrein-kinin system plays a pivotal role in suppressing the early development of hypertension by allowing sodium excretion.

V. Novel approaches to development of drugs against hypertension

A large variety of anti-hypertensive drugs are available for controlling hypertension, for example, diuretics, calcium entry blockers, adrenergic \( \alpha_1 \)-receptor antagonists and \( \beta \)-receptor antagonists, and ACE inhibitors. Nevertheless, no drugs have been developed to “prevent” hypertension, since the mechanism of development of essential hypertension is not known. We propose that the renal kallikrein-kinin system may play a suppressive role in the initial stage of hypertension, and that reduction of its activity may trigger the development of hypertension through sodium accumulation in animals predisposed to such accumulation. Therefore, some of the drugs that potentiate the renal kallikrein-kinin system may be useful for preventing essential hypertension. Inhibitors of urinary kininases are regarded as such drugs. Another type of drug may accelerate the secretion of renal kallikrein from the renal connecting duct.

1. Inhibition of kinin degradation
   a. Renal kininase inhibitors

Ebelactone B is isolated from the culture medium of *Actinomycetes* and selectively inhibits the activity of carboxypeptidase Y-like exopeptidase in rat urine as well as carboxypeptidase Y from yeast without inhibiting carboxypeptidases A and B or other kininases in the plasma and urine (111). Administration of ebelactone B to anesthetized rats caused diuresis and natriuresis in parallel with increased secretion of urinary kinin. This diuresis and natriuresis due to ebelactone B are blocked by the BK antagonist Hoe 140 (211). In DOCA-salt hypertension rats (185), subcutaneous infusion of lisinopril with a mini-osmotic pump for one week from 8 weeks of age does not reduce the systemic blood pressure, since renin has been suppressed in this model. In contrast, the high blood pressure is suppressed by subcutaneous infusion of ebelactone B (211) (Fig. 20). The urinary sodium excretion of normal BN-Ki rats was increased and urine volume tended to be increased in the BN-Ki rats. Mutant kininogen-deficient BN-Ka rats rapidly developed hypertension on the same treatment, and their systemic blood pressure leveled off 2 weeks after the onset of the treatment (at 9 weeks of age), but neither ebelactone B nor lisinopril had any effect, since no kinin was generated in the urine (211). Poststatin, which is isolated from the fermentation broth of *Streptomyces viridochromogens* MH534-30F3, inhibits all kininase activity in rat urine (108). Treatment of rats in DOCA-salt hypertension with poststatin also reduced the high blood pressure during this treatment (212).

b. Inhibitors of neutral endopeptidase

NEP is another major kininase in rat urine (110), but is also reported to be a proteinase for hydrolysis of atrial natriuretic peptide (or factor) (ANP) and enkephalins (213) and significantly contributes to the extrarenal metabolism of ANP (214). It has also been reported that a peptidase sensitive to phosphoramidon, an inhibitor of NEP, is present in the pig kidney microvillar membrane (215). NEP is present in high concentration in the glomeruli and brush borders of the proximal tubules of the kidney (216). In fact, NEP is responsible for 68% of the total kininases in the rat. Phosphoramidon decreased the total kininase activity by 77% and increased kinin excretion by 73%, urine volume by 15% and urinary excretion of sodium by 37% without changing the systemic blood pressure, renal blood flow, the glomerular filtration rate or urinary excretion of potassium (217). Many inhibitors of NEP have been developed. They increase the
endogenous ANP plasma level in normal volunteers and in experimental animals (218–221), or in congestive heart failure models (222, 223) and in cirrhotic patients with ascites (224), in association with an increase in urine volume and mean urinary sodium excretion. NEP does not contribute to the kinin hydrolysis in plasma (225).

It is interesting to know whether NEP inhibitors suppress the systemic blood pressure in hypertensive models and hypertensive patients. NEP inhibitors, candoxatrilat, or its prodrug candoxatril, and SCH 34826 ((S)-N-[N-[1-[2,2-dimethyl-1,3-dioxolan-4-yl]methoxy]carbonyl]-2-phenylethyl]-L-phenylalanine), reduced the systolic blood pressure of one-kidney DOCA-salt hypertensive rats by 30–40% (215, 226, 227) for 3 hr with increased urine volume output and renal urinary sodium excretion. NEP does not contribute to the kinin hydrolysis in plasma (225).

Fig. 20. Effects of ebelactone B and lisinopril on the developmental stage of deoxycorticosterone acetate-salt hypertension. Values (systolic blood pressure) are means ± SEM of the number (n) of rats. After uninephrectomy at 7 weeks of age, deoxycorticosterone acetate (5 mg/kg, s.c.) was administered once a week. From 8 weeks of age, ebelactone B (5, 15 mg/kg/day) or lisinopril (5 mg/kg/day) was administered (s.c.) for a week to deoxycorticosterone acetate-salt treated normal BN-Ki rats and kininogen-deficient BN-Ka rats. Values from rats receiving ebelactone B or lisinopril were compared with those of rats receiving vehicle at the same time. *P<0.05.

(quoted from Ref. 211 with permission)
binant ANP(1–28) in the plasma of Dahl salt-sensitive rats is degraded to α-recombinant ANP(1–25) in urine, and candoxatril inhibits this degradation (232), indicating the presence of NEP in the kidney (233, 234). Indeed, NEP is found in high concentrations in the kidney, liver and lung (235). This natriuresis by ANP was completely abolished by a BK antagonist (236), but it is reported that no BK antagonist contributes to the antihypertensive response to NEP inhibition (228, 229, 237). Infusion of an NEP inhibitor, UK73967 (3-[I-[[4-carboxycyclohexyl]amino][carbonyl]cyclopentyl]-2-[((methoxyethoxy)methyl]propionic acid) (10 mg/kg), into anesthetized normotensive rats significantly decreased NEP activity and increased kinin, urine volume and urinary sodium excretion levels, but did not induce any significant increase in plasma ANP. Simultaneous administration of Hoe 140 canceled the increases of urine volume and urinary sodium excretion caused by UK73967 (238). These results indicate that NEP may play some role in the kidney so that its inhibition induces natriuresis, probably through inhibition of kinin degradation. These results may support our findings that NEP is one of the major kininases contributing to kinin degradation in rat urine (110). Sixty percent of the total kininase activities in human urine are attributed to NEP (239). Thus, it is important to see the effects of NEP inhibitors in essential hypertension.

In clinical studies with NEP inhibitors in hypertensive patients, the results were not always consistent on the role of ANP: Candoxatril (10, 50 and 200 mg) raised the plasma ANP concentrations similarly at all three doses, but only the highest dose induced significant natriuresis without changes in blood pressure and heart rate (240). Candoxatril also increased the plasma ANP levels in hypertensive patients in a sodium-related manner. Urinary sodium excretion was increased up to 6 hr after drug administration, but no difference from normotensive subjects was observed in urinary cGMP excretion (241). In contrast, SCH 42495 (N-[2(S)-(acetylthiomethyl)-3-(2-methyl-phenyl)-1-oxopropyl]-l-methionine ethyl ester) increased the plasma cGMP level in positive correlation

![Fig. 21. Effect of Hoe 140, a bradykinin B2-antagonist, on the increases in urine volume (A) and urinary excretions of active kallikrein (B), sodium (C), chloride (D), potassium (E) and creatinine (F) induced by intravenous infusion of oxytocin (OT). OT was infused at the rate of 30 nmol/kg/30 min, and Hoe 140 was infused at the rate of 4.5 mg/kg/90 min as shown in the scale below. The value represents the means ± S.E.M. Each value of the Hoe 140-treated group (■) was compared with that of the Hoe 140 non-treated group (OT-infused group) (●) (P< 0.05, **P< 0.01) or the vehicle-infused group (□) (P< 0.05, ***P< 0.001) at the same time period. In the urinary kallikrein analysis, the values during infusion of OT were compared with that at the time of 15 min (P< 0.05, ***P< 0.001). ●: n= 6 for A and B, n= 5 for C–E, n= 4 for F, ■: n= 5 for A–F, □: n= 5 for A–E, n= 4 for F. (quoted from Ref. 84 with permission)
with an increase in the plasma ANP level (242). A 28-day course of treatment of essential hypertensive patients with candoxatril (200 mg) did not induce either a fall in supine blood pressure or urinary excretion of more cGMP than was excreted by the placebo-treated patients (243). In salt-loaded volunteers, SCH 34826 (400–600 mg) significantly increased the urinary excretion of sodium, cGMP and ANP without causing changes in ANP and cGMP levels in the plasma, suggesting that ANP has specific renal effects in normal individuals after sodium loading (244). In low-renin essential hypertensive patients, SCH 34826 (400 mg, four times a day) significantly reduced supine systolic and diastolic blood pressures, but the urinary excretion of sodium and the urine volume was not altered (245). These results suggest that in human subjects, NEP is present in the renal tubules and that NEP inhibitors may induce natriuresis and diuresis by inhibition of NEP in the kidney, probably through the inhibition of kinin degradation in the renal tubules. Experiments should be carefully designed to provide clear evidence, since the renal kallikrein-kinin system works only when sodium is liable to be accumulated in the body.

2. Enhanced kinin generation

One technique for raising the kinin concentration in the renal cortical collecting duct is to accelerate excretion of urinary kallikrein. Several stimuli for kallikrein secretion are known (84) (see section I, 3 a ii), including sodium deprivation, administration of DOCA or aldosterone, and potassium intake. These medical interventions could be used to accelerate urinary kallikrein secretion. We may add oxytocin can be added as another such accelerator. Oxytocin differs in its structure from vasopressin only by two amino acids, and it was reported (246) that the serum level of oxytocin in males (1.80 ± 0.07 pU/ml) is the same as that in non-pregnant females (1.71 ± 0.07 pU/ml); these results were confirmed by Leake et al. (247). The results definitely indicate that oxytocin plays a physiological role in both males and females, in addition to its induction of the uterine contractions of labor in females. As Fig. 21 indicates, in SD strain male rats, intravenous infusion of oxytocin under pentobarbital anesthesia increases the urine volume and urinary sodium excretion as well as kallikrein secretion (84). The increase in sodium excretion and urine volume due to oxytocin was markedly reduced by infusion of the BK B2-receptor antagonist Hoe 140. Thus, oxytocin may be a candidate for a urinary kallikrein releaser, although it has own weak natriuretic activity.

Short summary: Besides many known anti-hypertensive drugs, an alternative approach against hypertension may be to increase the amount of urinary kinin. As the degradation pathway of BK by urine is quite different from that by plasma, inhibitors of urinary kininases may be a good candidate of this type of drugs. Ebelactone B, an inhibitor of carboxypeptidase Y, reduced the high blood pressure in the rat DOCA-salt hypertension model, whereas ACE inhibitors are ineffective. Many inhibitors of NEP have been developed, but inhibition of ANP degradation has been focused on, while that of BK degradation in urine is ignored. However, recent reports suggest that the site of action may be the kidney and the possibility that BK may be a target peptide for the hypertensive action cannot be excluded, since NEP is one of urinary kininases in rats and humans. Secondly, if congenitally hypertensive animals as well as patients with essential hypertension secrete less kallikrein, agents that accelerate secretion of urinary kallikrein may be another way to develop new drugs. The findings that oxytocin, which is an endogenous hypophysis hormone, accelerates secretion of urinary kallikrein may be interesting and provide a clue for the development of new anti-hypertensive drugs.

Conclusions

Along the nephrons from the connecting tubules to the collecting duct, the kidney is equipped with all the components of the kallikrein-kinin system. Kinins are known to inhibit sodium and chloride reabsorption in the collecting tubular cells through distributed B2-receptors and to increase the secretion of sodium and water. However, the system does not appear to be active in sodium and water excretion, unless there is a tendency for sodium to be accumulated in the body. Once this condition is met, however, kinin generated by renal kallikrein causes the excretion of sodium and water. Accordingly, reduction of kinin generation either by reduced excretion of renal kallikrein, as in SHR, or through a deficiency of LMW kininogen in the plasma, as in mutant BN-Ka rats, may cause accumulation of sodium in cells such as the erythrocytes and probably the vascular smooth muscle cells, and in body fluids, such as cerebrospinal fluid. Sodium accumulation in vascular smooth muscle cells shifts to the left the dose-response curve of arterioles for vasoconstrictors like norepinephrine and angiotensin II. In addition, sodium retention in the cerebrospinal fluid results in increased sympathetic discharge. The reduced excretion of urinary kallikrein in hypertensive patients as well as in hypertensive models, which has been discussed for a long period, has not been clearly established, but large-scale studies on genetic and environmental determinants have revealed that low kallikrein homozygotes have a high risk of hypertension, and heterozygotes for kallikrein genotypes with low potassium intake are also
highly susceptible to hypertension. When these findings are taken together, the cause of essential hypertension may be simply explained by decreased sodium excretion in the kidney and sodium accumulation in the body. The contribution of the renal kallikrein-kinin system may be limited in the early stage of the development of hypertension, as seen in DOCA-salt hypertension models, but drugs that enhance the renal kallikrein-kinin system, such as urinary kininase inhibitors or accelerators of urinary kallikrein excretion, may be useful as a new approach for controlling of essential hypertension.

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