

## Review

## A Missing Link Between a High Salt Intake and Blood Pressure Increase

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**Abstract.** It is widely accepted that a high sodium intake triggers blood pressure rise. However, only one-third of the normotensive subjects were reported to show salt-sensitivity in their blood pressure. Many factors have been proposed as causes of salt-sensitive hypertension, but none of them provides a satisfactory explanation. We propose, on the basis of accumulated data, that the reduced activity of the kallikrein-kinin system in the kidney may provide this link. Renal kallikrein is secreted by the distal connecting tubular cells and all kallikrein-kinin system components are distributed along the collecting ducts in the distal nephron. Bradykinin generated is immediately destroyed by carboxypeptidase Y-like exopeptidase and neutral endopeptidase, both quite independent from the kininases in plasma, such as angiotensin converting enzyme. The salt-sensitivity of the blood pressure depends largely upon ethnicity and potassium intake. Interestingly, potassium and ATP-sensitive potassium ( $K_{ATP}$ ) channel blockers accelerate renal kallikrein secretion and suppress blood pressure rises in animal hypertension models. Measurement of urinary kallikrein may become necessary in salt-sensitive normotensive and hypertensive subjects. Furthermore, pharmaceutical development of renal kallikrein releasers, such as  $K_{ATP}$  channel blockers, and renal kininase inhibitors, such as ebelactone B, may lead to the development of novel antihypertensive drugs.

**Keywords:** salt-sensitivity, kallikrein, potassium, African-American, ebelactone B

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## I. Introduction

The "INTERSALT" population study is a collaborative study of the relationship between blood pressure (BP) and sodium and potassium intake in more than 50 population samples in 32 countries (1). In this study, significant positive associations were observed between BP and 24-h sodium excretion, body mass index, and alcohol intake. In contrast, significant negative associations were seen between BP and potassium excretion (2). The Dietary Approaches to Stop Hypertension (DASH) diet, which emphasizes fruits, vegetables, low-fat dairy products, whole grains, poultry, fish, and nuts, with only small amounts of red meat and sugar-containing beverage, lowers BP. Ingestion of more fruits and vegetables means the intake of more potassium. Combining the DASH diet with lower sodium intake further lowers BP (3). However, the DASH-Sodium Trial investigators determined that identifying salt responders was very difficult. On the basis of these findings (4), the authors supported sodium restriction only in the general population.

The relationship between a high salt intake and BP rise has also been repeatedly reviewed and discussed by Weinberger (5–9). It was verified in animals that salt sensitivity is essentially related to the kidney and is regulated by genetic factors, as seen in Dahl salt-sensitive and salt-resistant rats (see review (10)). Now, it can be stated that the relationship of high salt intake to BP increase has been convincingly established by epidemiological, observational, interventional, animal, and genetic studies. However, the BP responses of humans to alteration of sodium and extracellular fluid balance are heterogeneous among normotensive and hypertensive subjects. In addition, the mechanisms whereby high BP is reached through a high intake of salt are obscure.

African-Americans are more salt-sensitive than whites. Furthermore, the sodium sensitivity of BP increases significantly with increasing age in the entire population. This relationship was more striking in

hypertensive subjects, in whom a progressive increase in salt sensitivity with the passage of decades was seen, than in a normotensive group in whom salt sensitivity was not observed until the sixth decade (11).

## II. One-third of normotensive subjects are salt-sensitive

### 1. Definition of salt-sensitivity of the BP

The definition of the sodium sensitivity of BP has generally been based on the differences between the BP after a low sodium intake, such as 9 mmol/day (ca. 0.5 g of NaCl), and that after a high sodium intake, such as 249 mmol/day (12). A difference of at least 10% between the low- and high-salt mean arterial pressures is regarded as indicating salt-sensitivity, and a smaller difference as identifying a non-salt sensitive subject, after salt loading (12).

Weinberger's group used another stratagem for defining the salt sensitivity of the BP (13). BP was measured after an intravenous infusion of 2 liters of normal (0.9%) saline (308 mmol or 18 g of NaCl) for 4 h, and on the next day, it was measured again after sodium and volume depletion induced by a low sodium diet (10 mmol) and furosemide administration, in 378 healthy volunteers and 198 subjects with essential hypertension (13). Those in whom mean arterial BP decreased by at least 10 mmHg after sodium and volume depletion were considered sodium-sensitive, and those with a decrease of 5 mmHg or less (including an increase in pressure) were considered sodium-resistant. Subjects with a decrease in BP between 6 and 9 mmHg were classified as indeterminate. The responses of the BP were heterogeneous and formed a Gaussian distribution in both normotensive and hypertensive groups. It was found that 26% of the normotensive subjects were salt-sensitive and 58% salt-resistant, whereas in the hypertensive group, 51% were sensitive and 33% were resistant. Blacks have been shown consistently to have a greater frequency of salt sensitivity than whites (5). Weinberger observed that 73% of black hypertensive

patients were salt-sensitive, compared with 56% of a white hypertensive group. However, in the normotensive population, the frequency of salt sensitivity among blacks (36%) was similar to that seen among whites (29%). Plasma renin activity (low, normal, high) did not predict the sodium response. In both groups, the sodium-sensitive individuals were significantly older and had lower baselines of renin values than sodium-resistant subjects. Subpopulations with increased frequencies of salt sensitivity have been shown to be associated with high-BP, higher age, and African-American ethnicity.

In the Japanese population, a positive family history of hypertension was more frequently reported among salt-sensitive subjects (14).

Thus, abundant evidences from epidemiological and interventional studies have established heterogeneity in the BP responses of humans in both normotensive and hypertensive subjects to alterations in sodium and extracellular fluid balance and some consistent demographic factors, such as older age and black race, have been shown to be associated with an increased frequency of salt sensitivity (5, 13).

## 2. Salt-sensitivity of African-Americans and potassium

The prevalence of salt sensitivity (53.5% vs 51%) and salt resistance (25.3% vs 30.0%) in the participants was reported to be similar in African-American and white women (15). However, a greater mean BP increase with salt loading was seen in African-American vs white hypertensives, but not between the normotensive women. In erythrocytes,  $[Na^+]_i$ ,  $[Ca^{2+}]_i$ , and the ratios of  $[Na^+]_i$  to  $[K^+]_i$  and of  $[Ca^{2+}]_i$  to  $[Mg^{2+}]_i$  were higher in African-Americans and were positively correlated with salt responsiveness, but this was not observed in white women (15). Sodium pump activity was similar in the white and black groups, although the changes in maximal activity tended to vary inversely with salt sensitivity in African-Americans (15).

Thirty-eight healthy normotensive men (24 blacks, 14 whites) took a basal diet low in sodium (15 mmol/day) and marginally deficient in potassium (30 mmol/day) for 6 weeks. Throughout the last 4 weeks, loading of NaCl (250 mmol/day) was carried out. Throughout the last 3 weeks, potassium bicarbonate was added to give mid- or high-normal levels, namely 70 and 120 mmol/day. When dietary potassium was low at 30 mmol/day, salt loading induced a mean increase in the BP only in blacks ( $P < 0.001$ ), and salt sensitivity occurred in most blacks but not in whites (79% vs 36%). Supplementing potassium to a level of only 70 mmol/day attenuated moderate salt sensitivity similarly

in blacks and whites. Supplementation of potassium to 120 mmol/day suppressed the frequency and severity of salt sensitivity in blacks to levels similar to those observed in whites. Thus, it can be concluded that in most normotensive black men, but not in white men, salt sensitivity occurred when dietary potassium was even marginally deficient, but was dose-dependently suppressed when dietary potassium increased within its normal range (16).

Black adolescents, who showed a significant increase in urinary sodium excretion, were identified as salt-sensitive if their mean BP increase was  $\geq 5$  mmHg between the low-sodium (54 mmol/day) and the high-sodium (150 mmol/day) diet periods (17). A significantly greater percentage of salt-sensitive (44%) than salt-resistant (7%) subjects were "non-dippers" (no drop in nocturnal diastolic pressure) on the basis of diastolic BP classification ( $P < 0.04$ ). After the dietary intervention, all of the salt-sensitive subjects in the high-potassium group achieved "dipper" status as a result of a drop in nocturnal diastolic pressure.

In the steady state, urinary potassium represents dietary potassium intake (18). Blacks excreted less sodium and potassium in the 24-h urine collection than whites. After volume expansion with 0.9% NaCl for 4 h and volume contraction with low sodium and furosemide, blacks and subjects of 40 years of age or more excreted less sodium than whites and subjects less than 40 years. Blacks excreted less potassium and had higher BP than whites after saline administration and also showed greater suppression of plasma renin activity than whites 24 h after saline administration (19).

Blacks appear, on average, to retain more sodium than whites, but increased sodium retention in blacks does not appear to be secondary to increased production of either aldosterone, deoxycorticosterone, cortisol, or 18-hydrocortisol (20).

Blacks had higher BP after sodium loading than whites. Sodium loading caused a significant kaliuresis that was greater in whites than in blacks (21).

Many clinical trials have shown that increasing potassium intake lowers BP both in hypertensives and, to lesser extent, in normotensives (22). Potassium citrate in fruits and vegetables has lowering effect on BP similar to that of potassium chloride. In this context, it is interesting that Seventh-Day Adventist vegetarians showed significantly less hypertension and lower BP compared with Mormon omnivores and that healthy normotensive omnivores living on a lacto-ovo-vegetarian diet showed a reversible reduction of 5 to 6 mmHg in systolic and 2 to 3 mmHg in diastolic pressure over a 6-week period (23). Supplemental  $KHCO_3$  abolished the pressor effect of NaCl in salt-sensitive subjects,

but renal blood flow was unaffected, and the glomerular filtration rate and filtration fraction decreased (24).

### III. How is a high salt intake linked to BP?

A number of studies have been conducted in an attempt to gain insight into the mechanisms possibly responsible for the BP increases seen with a high salt intake. However, the mechanisms responsible for increased BP in response to a high dietary sodium intake in salt-sensitive patients with essential hypertension are only partially understood.

#### 1. Renin activity

Several studies indicated that the renin-angiotensin system may be less stimulated or suppressed when the BP falls as a result of reduction in sodium intake or extracellular volume than in subjects in whom little change or even a rise in BP is observed (25). It is claimed that suppression of the renin-angiotensin system can protect against a rise in BP when sodium intake is increased (9). Thus, the renin-angiotensin-aldosterone system can be excluded from the factors involved in BP rise.

#### 2. Volume expansion

Salt-induced, experimental hypertension is characterized by early increases in fluid volumes and cardiac output, which subsequently revert toward normal. The peripheral resistance initially decreases slightly and then continues to increase. Thus, the arterial BP is subsequently maintained by this elevated peripheral resistance (26). This implies that the main component maintaining BP elevation is the peripheral resistance or contraction of the arteriolar smooth muscles. The effect of a high salt intake on BP is enhanced by reductions in renal mass.

The elevation of BP due to intravenous infusion of 2 liters of 0.9% NaCl for 4 h, introduced by Weinberger's group (13), may be caused mainly by a large amount of NaCl (308 mmol) during the short period of 4 h.

White human subjects with normal renin hypertension have an absolute increase in intracellular fluid (red cell mass), compared with their age-matched peers, whereas subjects with low renin hypertension exhibit no volume differences from age-matched subjects with normal renin hypertension (27).

#### 3. Enhanced activity of the sympathetic nervous system

Hypertensive patients show higher activity of the sympathetic nervous system (28). The elevated sympathetic nerve activity may cause the salt-induced hyper-

tension to develop. However, there remains a lack of consensus on the role of the sympathetic nervous system in essential hypertension, which may reflect several factors (28). Nevertheless, there is an important report that intracisternal bolus injections of increasing concentrations of NaCl directly into the *cisterna magna* of Sprague-Dawley (SD) strain rats enhances the discharge of the sympathetic nerves in a concentration-dependent manner and increases systolic blood pressure (SBP) (29). Even if the enhanced sympathetic nerve activity may contribute to the vasoconstriction, sodium accumulation in the cerebrospinal fluid (CSF) should be ranked higher.

#### 4. Ion exchangers

During high salt loading, an increase in intra-cellular (erythrocyte) calcium and sodium concentrations and a reduction in magnesium concentration were observed primarily in salt-sensitive subjects (30).

Elevated erythrocyte sodium-lithium ( $\text{Na}^+\text{-Li}^+$ ) counter-transport (CT) is well known among intermediate phenotypes in human hypertension because pedigree studies have shown support of major gene inheritance of the trait (31). Striking similarities between this CT and  $\text{Na}^+\text{-H}^+$  exchange suggest that the mutation at the  $\text{Na}^+\text{-H}^+$  antiporter gene locus might result in elevated  $\text{Na}^+\text{-Li}^+$  CT and contribute to the subsequent pathogenesis of hypertension. However, the results of linkage analysis excluded the  $\text{Na}^+\text{-H}^+$  antiporter gene locus as a candidate gene (32).

Increased activity of epithelial sodium channels in the distal tubules of the kidney is the cause of high BP in Liddle's syndrome. Since sodium channels similar to renal channels are present in the nasal epithelium, the transnasal potential difference was measured in white hypertensive patients after amiloride application. There was no difference in maximum potential between hypertensive and normotensive subjects (33).

In attempt to link the red blood cell transport abnormality to an alteration in renal tubular sodium handling, both erythrocyte  $\text{Na}^+\text{-Li}^+$  CT in vitro and lithium clearance in vivo were studied in normotensive and hypertensive humans. The hypertensive patients had an increased  $\text{Na}^+\text{-Li}^+$  CT. The fractional excretion of lithium was higher in those with elevated BP, despite similar baseline values for the fractional excretion of sodium between normotensive and hypertensive individuals (34). Moreover, volume expansion caused a greater natriuresis and greater increase in fractional excretion of lithium among the hypertensive patients. It is suggested that hypertensive patients do not have increased proximal tubular sodium reabsorption and that the exaggerated natriuresis often observed in hyper-

tension is a result of increased distal tubular sodium delivery (34). These conclusions were also confirmed in another paper (35).

Regarding ion transport in erythrocytes, the total population of patients with essential hypertension is heterogeneous and includes a subgroup of higher  $\text{Na}^+$ - $\text{Li}^+$  CT flux with normal  $\text{Na}^+$ - $\text{K}^+$  cotransport (COT) function and one of abnormal  $\text{Na}^+$ - $\text{K}^+$  COT function with normal  $\text{Na}^+$ - $\text{Li}^+$  CT flux (36). In hypertensive patients,  $\text{Na}^+$ - $\text{Li}^+$  CT is elevated compared with that in normal subjects, and whites have a higher level of  $\text{Na}^+$ - $\text{Li}^+$  CT than blacks (34).

### 5. $\alpha$ -Adducin

It was reported that Milano hypertensive strain (MHS) rats showed faster outward  $\text{Na}^+$ - $\text{K}^+$ - $\text{Cl}^-$  COT in erythrocytes than did Milan normotensive strain rats, so that MHS rats had a smaller volume of erythrocytes with a lower sodium content (37).

In an Italian study (38), 490 hypertensive patients and 176 normotensive subjects in Sassari and 468 hypertensive patients and 181 normotensive subjects in Milan were genotyped for  $\alpha$ -adducin Gly460Trp polymorphism. The  $\alpha$ -adducin 460Trp allele was more frequently found in hypertensive patients than in normotensive subjects in the Milan population ( $P = 0.0199$ ), but the difference was not significant in Sassari.

The frequency of the 460Trp allele was 19%, and 9 of 279 subjects (3.2%) were homozygous for that allele (39). The SBP response to change in dietary sodium was significantly greater in subjects homozygous for the 460Trp polymorphism ( $25 \pm 2$  mmHg) than in subjects heterozygous for 460Trp ( $12 \pm 2$  mmHg) or homozygous for the 460Gly allele ( $14 \pm 1$  mmHg). Intracellular sodium content and  $\text{Na}^+$ - $\text{Li}^+$  CT in erythrocytes and renal fractional excretion of sodium were significantly decreased in those who were homozygous for the 460Trp polymorphism.

It was reported (40) that in the Japanese population of 2,902 subjects (the Shigaraki study), there was no correlation between eating salty food and combined angiotensinogen and adducin polymorphisms.

### 6. Nitric oxide

Increasing dietary sodium chloride increased nitric oxide (NO) activity in salt-resistant rats, but not in salt-sensitive rats. Exogenous L-arginine, the substrate for NO synthesis, decreased the BP of salt-sensitive rats to normotensive levels, which were made hypertensive for 2 weeks by 8% NaCl chow, but did not change the BP of salt-resistant rats (41). Normotensive rats fed a diet containing 8% NaCl produced greater amounts of total and active transforming growth factor- $\beta$  1 and NO (42).

However, it was reported that the genes of the urea cycle/arginine synthesis are unlikely to be involved in salt-sensitive hypertension in the Dahl/Rapp strain (43). A new coding variant of the endothelial NO synthase gene, Glu298Asp, showed a strong association with essential hypertension (Kyoto: odds ratio, 2.3 and Kumamoto: odds ratio, 2.4). The allele frequencies of 298Asp in hypertensive subjects were significantly higher than those in normotensive subjects in both groups (44). The involvement of the NO system in salt-sensitive hypertension still remains to be established.

Many factors have been proposed for the pathogenesis of salt-sensitive hypertension, but it cannot be concluded that they sufficiently explain the mechanisms of the elevated BP.

## IV. Reduced function of the kallikrein-kinin system in the kidney: is it possible that the reduced function of this system links a high salt intake to the elevated BP? — a proposed hypothesis

### 1. Salt sensitivity and reduced urinary kallikrein excretion in humans

A small-scale study was performed with 25 normotensive male and female subjects without any familial history of hypertension. They were divided into three groups on the basis of urinary kallikrein excretion (low, normal, and high) (45). After sodium loading, the urinary excretion of active renal kallikrein decreased in all three groups to the same degree while sodium excretion increased. SBP increased significantly in the low-kallikrein group, but remained unchanged in the normal-kallikrein group, and showed a tendency to decrease in the high-kallikrein group (low  $4.6 \pm 1.6$ ,  $P < 0.01$ ; normal  $+1.2 \pm 2.8$ ; high  $-2.1 \pm 2.1$  mmHg; low vs high:  $P < 0.0025$ ). Considering all groups together, there was a significant linear inverse relation between the change in BP during sodium loading and the urinary kallikrein excretion at maximum sodium restriction (SBP  $r = -0.4354$ ,  $P < 0.05$ ).

With a relatively small group of 37 male hypertensive patients, a randomized, crossover, double-blind study was performed (46). The patients were classified as salt-sensitive, when diastolic BP changes of 10 mmHg or more occurred after NaCl intake in both the period of low (40 mmol NaCl per day for 2 weeks) and that of high (240 mmol NaCl per day for 2 weeks). Nineteen hypertensive patients were salt-sensitive, while 18 were classified as salt-resistant. The urinary excretion of active kallikrein was significantly lower ( $P < 0.0001$ ) in salt-sensitive patients ( $0.51 \pm 0.36$  U/24 h) than in salt-resistant patients ( $1.28 \pm 0.48$  U/24 h).

Early in 1934 it was reported by Elliot and Nuzum

that hypertensive patients without clinically apparent renal disease showed lower urinary kallikrein levels than did normotensive subjects (47). In 1971, Margolius et al. reported lower levels of urinary kallikrein in patients with essential hypertension than in a control population (48). Since this study, a large number of other reports have been published in human hypertensives and in animal models of hypertension, indicating that urinary kallikrein is lower in hypertension (see review (10)). However, this notion has been thrown into confusion by the matter of ethnicity. Namely, both black and white subjects with essential hypertension excrete less kallikrein in their urine than do their respective controls, but the mean value in normotensive blacks is lower than in normotensive whites and is not different from that in hypertensive whites during normal sodium intake (49). All groups had greater urinary kallikrein activity on a low-sodium diet than on an unrestricted sodium intake, but the increase in black hypertensives was small.

Segregation analysis of a large number of Utah pedigrees, covering 1.2 million subjects as well as of 140,000 Utah death certificates over a 20-year period, was carried out to elucidate the genetic environmental determinants of hypertension, lipid abnormalities, and coronary arterial diseases (50). According to that author's observation, kallikrein levels in approximately

30% of the population are low in "low homozygotes", who have a high risk of hypertension (Fig. 1). It is interesting that this percentage is close to that of 26% of "salt-sensitive" population seen in the normotensive subjects, as was mentioned above. Are these coincidental?

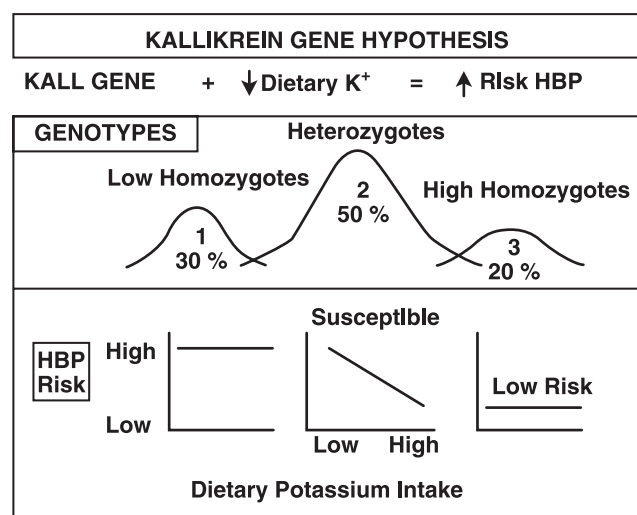
Approximately 20% of the population is "high homozygotes" who have a low risk of hypertension whatever their potassium intake. On the basis of their observation, Williams et al. (50) proposed the following hypothesis: approximately 50%, who are heterozygous for this single-gene trait, would be at high risk of hypertension when they ingest a low-potassium diet, whereas a high-potassium intake would reduce the risk of hypertension. This hypothesis is quite interesting, since suppression of hypertension with a potassium intake has been repeatedly discussed.

## 2. Are the rats that carry the reduced activity of the renal kallikrein-kinin system really salt-sensitive?

### 1) Lack of bradykinin generation in congenitally kininogen-deficient rats

The hypotensive peptide bradykinin (BK) is a major peptide of this group. BK, kallidin, and methionyl-lysyl-BK are found in the plasma and are generally called plasma kinins. They are generated by enzyme kallikreins from high molecular weight (HMW) and low molecular weight (LMW) kininogens.

Lack of kinin generation in the kidney can be induced in rats that are congenitally deficient of both HMW and LMW kininogens (precursors of kinins) in the plasma. This strain of rat was designated as Brown Norway Katholiek (BN-Ka) rats (51), which are almost entirely incapable of excreting kinins in the urine (52, 53). These mutant BN-Ka rats have the ability to produce both kininogens in the liver. However, they cannot release kininogens into the blood stream because of the point mutation of Ala<sup>163</sup> to threonine in the common heavy chain in the structure of both kininogens (54). Mutant BN-Ka rats were compared with normal rats of the same strain, which were designated BN-Kitasato (BN-Ki) rats (51). The plasma concentrations of HMW and LMW kininogens in the normal BN-Ki rats were  $15.8 \pm 0.8$  and  $8.9 \pm 0.6$  ng BK equivalent per mg plasma protein, respectively, and the concentrations were approximately the same as those in the SD strain of rats. In contrast, the plasma levels of HMW kininogens in mutant BN-Ka rats at 7 weeks of age were below the detection limit, and those of LMW kininogens were also very low (55). The amount of free kinin excreted in the ureteral urine in normal 7-week-old BN-Ki rats was  $114.3 \pm 48.9$  ng BK per 24 h, but the amount was very low in mutant BN-Ka rats ( $6.1 \pm 0.6$  ng BK per 24 h) (55). The mRNAs of



**Fig. 1.** Diagram of the hypothesis that the degree of interaction between urinary kallikrein genotypes and potassium intake accounts for the degree of risk of hypertension. Kallikrein excretion is low in "low (kallikrein) homozygotes" that are associated with a high risk of hypertension. "High (kallikrein) homozygotes" are linked with a low risk of hypertension regardless of potassium intake. Approximately half of the general population with the heterozygous kallikrein genotype can have either a high or a low susceptibility to hypertension, depending on whether potassium intake is low or high, respectively. HBP, high blood pressure. Reproduced from ref. 50 with permission from American Journal of Hypertension Ltd. ©1993.

HMW and LMW kininogens and prekallikrein that are present in the liver of BN-Ka rats are of similar size and abundance, compared with those in control BN (BN-Orl) rats (56).

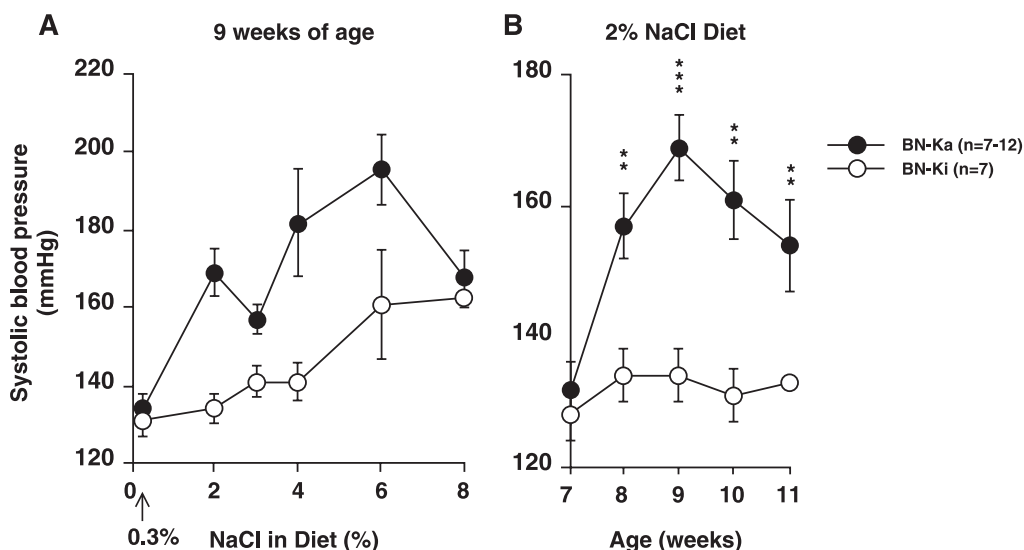
## 2) The kininogen-deficient rats are salt-sensitive

Changes in SBP during growth in mutant BN-Ka rats are the same as in normal BN-Ki rats, when they are fed a diet containing 0.3% NaCl and drink distilled water (53). An increase in the dietary concentration of NaCl up to 4% does not cause elevation of the SBP in normal BN-Ki rats, as measured by tail cuff plethysmography (Fig. 2A). In contrast, kininogens-deficient BN-Ka rats showed an increase in SBP while receiving only a 2% concentration of NaCl in their diet (Fig. 2: A and B) (55). In the deficient BN-Ka rats, the SBP increased to  $167 \pm 4$  mmHg within 2 weeks, whereas that of normal BN-Ki rats did not change during a 4-week-period ( $133 \pm 3$  mmHg). During the period of feeding with a 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 did normal BN-Ki rats (55), so that the tentatively calculated difference (water intake minus urine volume) was much greater in the deficient BN-Ka rats than that in the normal BN-Ki rats, which remained constant during the 4-week-period. Urine excretion of sodium also increased, but mutant BN-Ka rats excreted less than the normal BN-Ki rats. No difference was observed in urinary excretion of potassium and creatinine between normal BN-Ki rats and mutant BN-Ka rats.

The serum sodium levels of normal BN-Ki rats were constant, as expected. It is important to mention that despite the reduced excretion of sodium and water, the serum sodium levels in mutant BN-Ka rats tended to be higher, but the difference from those in normal BN-Ki rats was not statistically significant with the exception of the value in the 2nd week of the experiment, which was slightly higher than that of normal BN-Ki rats. Instead, 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 (55). Supplementation of LMW kininogen, by infusion for 7 days by means of a mini-osmotic pump implanted subcutaneously in the back of the kininogen-deficient BN-Ka rats fed 2% NaCl diet, lowered the SBP to control levels and caused increases in urinary kinin, sodium excretion, and urine volume. Thus, the causal relationship of the reduced urinary kinin with high BP can be verified. In addition, the subcutaneous infusion of HOE 140, a selective  $B_2$ -receptor antagonist, into the normal BN-Ki rats was accompanied by reduced excretion of urinary sodium and reduced urine volume (55).

It must be remembered that an intake of more than 4% of NaCl in the diet reduced excretion of active kallikrein, but not of prokallikrein, in the urine in both normal BN-Ki rats and deficient BN-Ka rats (55). Thus, the increase in SBP in normal BN-Ki rats resulting from NaCl concentrations of over 4% could have been caused by reduced excretion of urinary active kallikrein.

There is a report (57) that the tentative SBP rise in kininogen-deficient BN-Ka rats induced by a high salt



**Fig. 2.** Changes in systolic blood pressure in normal BN-Ki rats and kininogen-deficient BN-Ka rats given NaCl-loaded diet. Both strains of rats were fed 2% to 8% NaCl diets from the age of 7 weeks for 2 weeks (panel A) and a 2% NaCl diet between the ages of 7 and 11 weeks (panel B). Values are means ( $\pm$  S.E.M.) of 7 to 12 rats. Values in BN-Ka rats were compared with those in BN-Ki rats of the same age.  $**P < 0.01$ ,  $***P < 0.001$ . From ref. 162 with permission.

diet or by infusion of a nonpressor dose of angiotensin II was not confirmed. A BK B<sub>2</sub> receptor antagonist, Hoe140, did not potentiate the pressor effect of a chronic subpressor dose of angiotensin II in male and female Wistar rats or that of a high salt diet (2%) plus nephrectomy in male Wistar rats. As we have stated (58), the description in the report was inaccurate, and some of the results for BP are questionable, and the authors of the original paper did not provide any substantial rebuttal against these criticisms.

### *3) BK B<sub>2</sub> receptor-gene-disrupted mice are also salt-sensitive*

The high sensitivity of the BP after salt loading was also observed in mice that are homozygous for the targeted disruption of the gene encoding BK B<sub>2</sub> receptor (B<sub>2</sub>-KO) (59–61). At the end of the 8-week period of ingesting a normal (0.2%) or high-Na<sup>+</sup> diet (3.15% in food + 1% saline in drinking water), control mice showed no difference in BP on either diet, whereas in B<sub>2</sub>-KO mice maintained on a high Na<sup>+</sup> diet, SBP was 15 mmHg higher than in B<sub>2</sub>-KO on a normal Na<sup>+</sup> diet ( $P < 0.01$ ) (61). Renal BP was reduced by 20% and renal vascular resistance was doubled in B<sub>2</sub>-KO mice on the high Na<sup>+</sup> diet. B<sub>2</sub>-KO mice developed salt-induced hypertension faster and had a higher end point BP ( $148 \pm 3.7$  vs  $133 \pm 3.1$  mmHg) (62). The B<sub>2</sub>-KO mice upregulated B<sub>1</sub> receptor (62). However, there is a report (63) that increasing dietary salt intake did not affect mean arterial BP and the heart rate of B<sub>2</sub>-KO mice, determined by a telemetric technique, and pressure-natriuresis curves, pressure-diuresis curves, renal blood flow, and glomerular filtration rate did not differ between B<sub>2</sub>-KO and normal 129v/J mice.

### *4) Increase in the arteriolar sensitivity to vasopressor substances after salt loading*

Intra-arterial infusion of either 0.15 or 0.3 M NaCl solution for 4 days into conscious, unrestrained normal BN-Ki rats through an indwelling catheter failed to increase the mean arterial pressure ( $112 \pm 3$  mmHg). Infusion of 0.15 M isotonic NaCl solution into kininogen-deficient BN-Ka rats did not change the mean arterial pressure either. In contrast, infusion of NaCl solution of twice the concentration (0.3 M) of NaCl solution into the deficient BN-Ka rats significantly increased the mean arterial pressure to  $127 \pm 3$  mmHg ( $P < 0.05$ ) one day after the start of infusion. Concomitant increases were observed in the sodium levels not only in the serum, but also in the erythrocytes and in the CSF (64). The dose-response curve of the arterioles for angiotensin II in the kininogen-deficient BN-Ka rats shifted to the left (10-fold increase) after infusion of

0.3 M NaCl, and the arteriolar sensitivity to norepinephrine also increased by a factor of 30 (64). In contrast, the sensitivity of the arterioles of normal BN-Ki rats to angiotensin II and norepinephrine did not change after the infusion of either 0.15 or 0.3 M NaCl. The increased sensitivity of the arterioles of the deficient BN-Ka rats may have been caused by sodium accumulation, not only in the erythrocytes, but also in the vascular smooth muscle cells, and in the CSF. A sodium accumulation in the CSF causes an increase in the activity of the sympathetic nervous system (29). This implies that the reduced renal kallikrein-kinin system links a high salt intake directly to elevated BP.

In the rat aorta and bovine tail artery, reducing external Na<sup>+</sup> and/or increasing internal Na<sup>+</sup> increased vascular reactivity to norepinephrine, to K<sup>+</sup>, and (in the rat aorta) to caffeine, and slowed relaxation; and marked reduction in the Na<sup>+</sup> gradient induced contraction (65). These effects appear to be the results of Ca<sup>2+</sup> movements mediated by Na<sup>+</sup>/Ca<sup>2+</sup> exchange.

In essential hypertension (66), salt-sensitive patients exhibited a greater BP response to norepinephrine than salt-resistant patients irrespective of the dietary sodium intake. On the other hand, the BP response to angiotensin II was greater in salt-sensitive than in salt-resistant patients during low but not during high sodium intake.

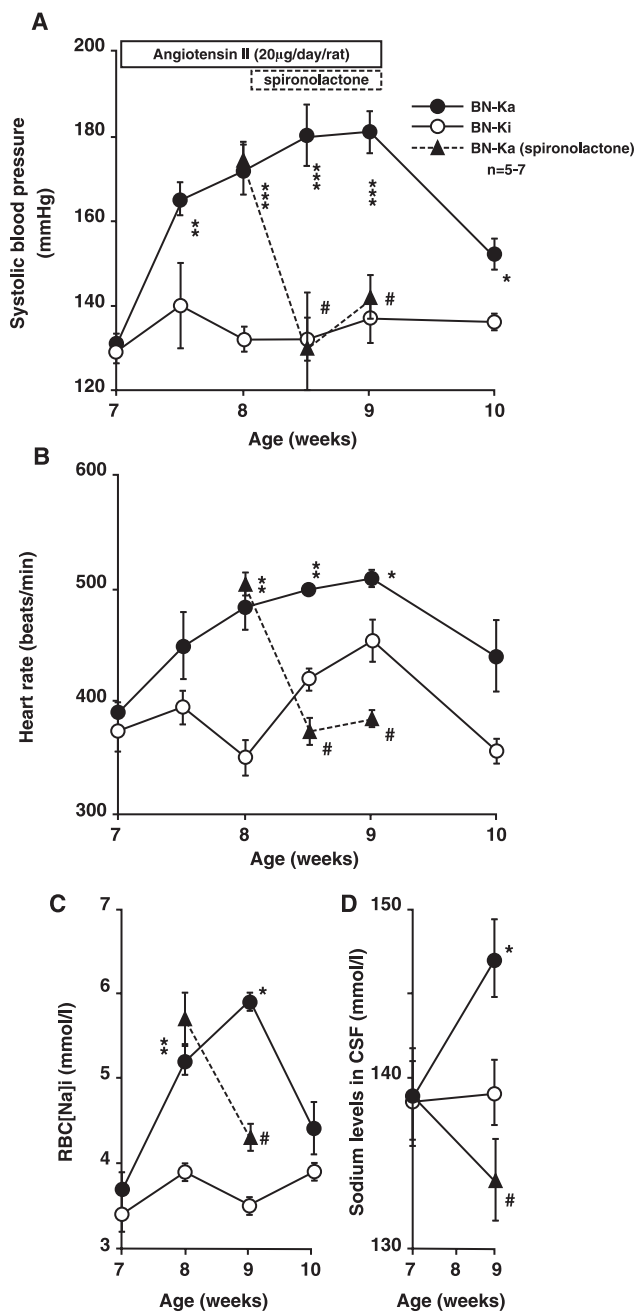
Large artery compliance in sodium-sensitive borderline hypertensives is lower than that in age-matched sodium-resistant subjects (67).

As an explanation of these phenomenon, inhibition of Na<sup>+</sup>-K<sup>+</sup> ATPase in vascular smooth muscles by a digitalis like factor has been proposed (68).

### *5) High BP due to sodium accumulation by non-pressor dose of angiotensin II in the kininogen-deficient BN-Ka rats*

The elevation of SBP was also attributable to sodium accumulation due to aldosterone release by angiotensin II, as shown in Fig. 3 (69). Subcutaneous infusion of a nonpressor dose (20 µg/day per rat) of angiotensin II in normal BN-Ki rats for 2 weeks with a mini-osmotic pump did not change the SBP. However, the same infusion in kininogen-deficient BN-Ka rats raised the SBP to  $180 \pm 8$  mmHg (Fig. 3), suggesting that hypertension is probably not attributable to direct vasoconstriction by this peptide. The heart rate and serum sodium levels were increased. The sodium levels in the erythrocytes and in the CSF were also increased. Urine volumes and urinary sodium excretion were not increased during angiotensin II infusion in deficient BN-Ka rats, but they were gradually increased in normal BN-Ki rats.





**Fig. 3.** Changes in (A) systolic blood pressures, (B) heart rates, (C) sodium concentrations in erythrocytes (RBC[Na]), and (D) sodium levels in cerebrospinal fluid (CSF) in normal BN-Ki rats and kininogen-deficient BN-Ka rats during infusion of a low dose of angiotensin II. Values are shown as means ( $\pm$  S.E.M.) ( $n = 5-7$ ). After blood pressure measurement at 7 weeks of age, Ang II ( $20 \mu\text{g/day}$  per rat) was subcutaneously (s.c.) infused for 2 weeks. Spironolactone ( $50 \text{ mg/day}$  per rat, s.c.) was given to Ang II-treated BN-Ka rats for 7 days. Values in BN-Ka rats were compared with those in normal BN-Ki rats at the same age. Closed circle: mutant BN-Ka, open circle: normal BN-Ki, closed triangle: BN-Ka (+ spironolactone). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Values in BN-Ka rats with spironolactone were compared with those in BN-Ka rats receiving Ang II alone. # $P < 0.05$ . Reproduced from ref. 10 with permission of Taylor & Francis Group ©2003.

As Fig. 3 indicates, simultaneous subcutaneous infusion of spironolactone (an aldosterone receptor antagonist) with angiotensin II in deficient BN-Ka rats in the second week of the angiotensin infusion period strikingly reduced the high SBP, the heart rate, and the sodium concentrations of erythrocytes and CSF to the levels in normal BN-Ki rats, indicating that the aldosterone released by the angiotensin II caused sodium accumulation in the body and sodium-accumulated hypertension was induced. There was no difference in increased urinary secretion of aldosterone between the two strains of rats.

In all hypertensive patients (WHO stages I and II) with low plasma renin activity, the sodium efflux rate constant of erythrocytes is decreased by volume expansion (one liter of 0.9% NaCl solution over 15–20 min). A positive correlation was observed between baseline plasma renin activity and change in the sodium efflux rate constant after volume expansion. At baseline, the relationship between plasma renin activity and intra-erythrocyte sodium content nearly reached statistical significance (70).

### 3. Genetically hypertensive rats and Dahl salt-sensitive rats

The initial rise of the SBP (up to the 5th week) in deoxycorticosterone acetate (DOCA)-salt-induced hypertension was prevented by the animal's own natural renal kallikrein-kinin system, since secretion of urinary kallikrein started to be increased and showed a peak at the 3rd week in the DOCA-salt rats (71). The SBP started to be increased in the normal BN-Ki rats after this peak secretion. As the kininogen-deficient BN-Ka rats cannot generate kinin, their SBP was increased from the beginning and was much greater during the first 5 weeks than in normal BN-Ki rats (53).

Reduced secretion of renal kallikrein was observed in Okamoto-Aoki's spontaneously hypertensive rats (SHR), particularly in weanlings (4-week-old) (72). Other genetically hypertensive rats, such as New Zealand strain rats (73), fawnhooded rats (74), and Milan hypertensive rats, also exhibit low excretion of urinary kallikrein (75).

In Dahl salt-sensitive rats that had been separated from Dahl salt-resistant rats, hypertension was induced by ingestion of excess sodium. Dahl salt-sensitive rats also have less urinary kallikrein activity than do Dahl salt-resistant rats (76, 77).

### 4. Polymorphisms of kallikrein and BK $B_2$ receptors

The restriction fragment length polymorphism (RFLP) of kallikrein may be a cause of raised SBP, since the recombinant inbred strain from SHR has its RFLP,

compared with that of normal Brown Norway strain (78). Nine single-nucleotide polymorphisms in the human kallikrein gene were identified and a significant decrease in urinary kallikrein activity was observed in the subjects heterozygous for the Arg53His polymorphisms, compared with the other subjects. However, none of the polymorphisms was associated with hypertension (79). It is not known whether these polymorphisms are related to the salt-sensitivity of the BP.

Ten alleles with length and nucleotide sequence variations were identified in the regulatory region of the human tissue kallikrein gene. There were nine alleles among the essential hypertension group and the control group, and the allele frequencies were found to be significantly different between the essential hypertension subjects and controls among the Chinese Han people (80).

The distribution of four different polymorphisms of the kinin B<sub>1</sub> and B<sub>2</sub> receptor genes was studied in a population of 120 normotensive and 77 hypertensive African-Americans (81). Among the polymorphisms analyzed, a potentially and functionally significant polymorphism in the core promoter of the kinin B<sub>2</sub> receptor (C-58-T transition) displayed greater prevalence of the C-58 allele in the hypertensive patients than in the control (0.72 vs 0.62,  $P=0.009$ ). This B<sub>2</sub> receptor promoter polymorphism may thus represent susceptibility to essential hypertension in African-Americans.

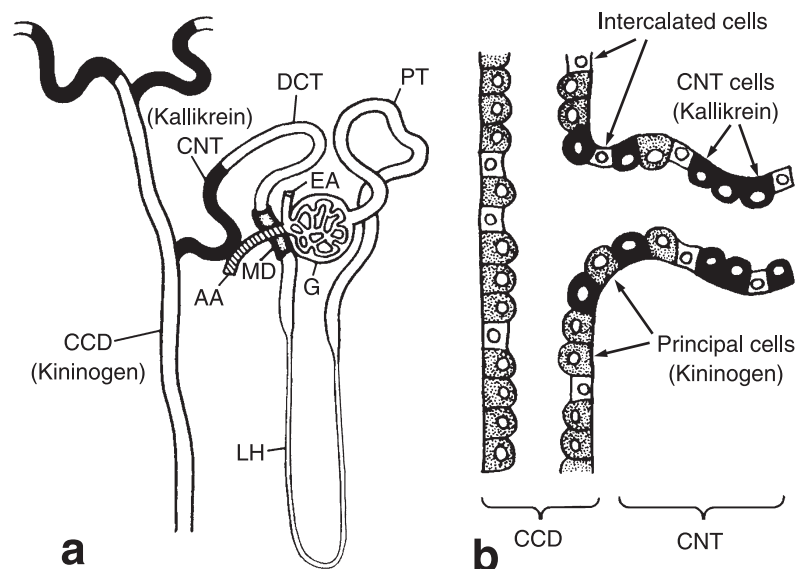
## V. Why is the renal kallikrein secreted in the distal nephron?

In the nephrons of the kidney, 90–95% of sodium is reabsorbed in the proximal portion and particularly in the loop of Henle before it reaches the distal nephron, and so studies on hypertension-related sodium retention have been focused in the renal proximal tubules. However, what is important, but not generally recognized, is that the cells of the connecting tubules (CNT) of the distal nephron have a crucial role of sodium handling in salt-sensitive hypertension.

All components of the tissue kallikrein-kinin system, including tissue kallikrein, LMW kininogen, BK B<sub>2</sub> receptors, renal kininases, and a tissue kallikrein inhibitor, are secreted and distributed in the epithelial cells in the distal tubules from the CNT cells to the collecting ducts (CD). The details of this will be discussed below.

### 1. Renal kallikrein

Two types of kallikreins exist in the body. Plasma kallikrein in plasma releases BK from its substrate, HMW kininogen. On the other hand, tissue kallikrein is contained in the cells of the glandular tissues, such as the pancreas, and in the cells of the kidney. This tissue kallikrein releases kallidin from LMW kininogen (See details in Review (10)). Renal kallikrein, one of the tissue kallikreins, will be discussed below.



**Fig. 4.** Diagram of the immunocytochemical localization of kallikrein and kininogen in the human nephron. a: Localization of kallikrein (CNT, closed) and kininogen (CCD) along the nephron. b: A schematic representation of the intermingled CNT cells and principal cells at the junction between CNT and CCD. AA, afferent arteriole; G, glomerulus; EA, efferent arterioles; PT, proximal tubule; LH, Loop of Henle; MD, Macula densa; DCT, distal convoluted tubule; CNT, connecting tubule; CCD, cortical collecting duct. Modified from Fig. 9 in ref. 87 with kind permission of Springer Science and Business Media ©1988.

Electron-micrographic studies have detected kallikrein only in the distal tubules (82, 83) and have revealed that renal kallikrein is localized in the granular cells of the CNT of the distal nephron. It is concentrated mainly on the luminal side of the cells and on both luminal and vascular sides of the nuclei and is to a lesser extent associated with the plasma membranes and basolateral infoldings (84, 85). Within the cells, kallikrein is distributed in the luminal membranes, basal membranes, rough endoplasmic reticulum, free polysomes, Golgi apparatus, and vesicles of the CNT cells. These findings suggest that it is actively synthesized in these particular cells (86). More interestingly, as Fig. 4b indicates, the CNT cells, which secrete tissue kallikrein, and the principal cells of the CD, which secrete its substrate, LMW kininogen, coexist side by side in the same transitional tubules (87). This indicates that kinins could be generated in the lumen of the CD adjacent to the site of kallikrein excretion. Additionally, the close anatomical contact between the kallikrein-containing cells and the afferent arterioles of the juxtaglomerular apparatus suggests that tissue kallikrein excreted on the basolateral side of the CNT might play some role in the regulation of the diameters of the afferent arterioles of the glomerulus (88).

Tissue kallikrein mRNA is expressed predominantly in the cells of the distal tubules and also in the vascular pole of the glomeruli (89) and in the CNT of the outer cortex (90). Another report (91) states that kallikrein is present in the granular peripolar cells of the glomeruli of the human kidney, but that its mRNA is not located at this site. Tissue kallikrein mRNA and protein are present in the walls of the large- and medium-sized blood vessels of the kidney (91), but its presence may be a general finding throughout the body. It was suggested (91) that the tissue kallikrein gene in the kidney may not be constitutively expressed but is induced in response to physiological and pathological stimuli, but this supposition needs to be confirmed.

## 2. LMW kininogen

Kininogen was detected in human urine (92, 93), but no intact HMW kininogen could be found in the kidney or urine (94). By the use of antibodies against the heavy (H) chain, which is common to HMW and LMW kininogens, LMW kininogen was isolated and the H-chain antigen was localized in the kidney, where it was diffusely distributed in the cells of the distal tubules and in the cortical and medullary CD. Precise studies (87) revealed localization in the principal cells of the CD of the immuno-reactive kininogen, which was restricted to the luminal portion of the principal cells (Fig. 4). The mRNA of LMW kininogen is expressed in the renal

cortex and the medulla (95), suggesting the biosynthesis of LMW kininogen in the distal tubules. In the mutant kininogen-deficient BN-Ka rats, it was reported that kininogen was synthesized in the tubular cells (96). It is probably not secreted into the lumen as it is in the liver in this particular strain of rats.

## 3. Kinins and kinin receptors

Early studies reported that human urine contains a mixture of BK, kallidin, and methionyl-lysyl-BK (97, 98); and large amounts of these kinins were later shown to be present in human urine (92). Rat urine also contains kinins. They must be generated in the kidney and in the urine itself since infusion of kallikrein did not increase the excretion of kinin in man (99, 100). Infusion of BK in man or into the renal artery of the dog also did not raise the urinary kinin level (100, 101) because of the very rapid inactivation of kinins in the proximal tubules.

Considerable knowledge of kinin receptors in the kidney has been accumulated. In accordance with the significant inhibition of the tubular efflux of  $^{22}\text{Na}$  in the distal nephron segment by BK administered into the lumen of late proximal convoluted tubules, the [ $^3\text{H}$ ]BK binding capacity is maximal in the cortical CD and the outer medullary CD, and small but significant in the glomerulus, the proximal straight tubules, the cortical thick ascending limb of the loop of Henle, and the distal convoluted tubule (102). Using chemically cross-linked conjugates of bovine serum albumin with a BK  $\text{B}_2$  agonist or the potent  $\text{B}_2$  antagonist HOE140 the receptor was found in the straight portions of the proximal tubules, in the thick ascending limb of the loop of Henle, in the CNT, and in the CD of the rat kidney (103). The  $\text{B}_2$  receptors are found in the luminal membranes, in the basal infoldings of the tubular cells, and in the smooth muscle cells of the cortical radial artery and of the afferent arterioles. The  $\text{B}_2$  receptors are colocalized in the CNT cell layers with kallikrein and in the CD cell layers with kininogens (103). The  $\text{B}_2$  receptor mRNA is colocalized with kininogen mRNA in the kidney, and the most intense signals are observed in the distal tubules and CD (104). The localization of  $\text{B}_2$  receptors in the CD may be compatible with the observation that BK inhibits the net sodium absorption in the CD (105). The  $\text{B}_1$  receptor gene was reported to be present in the kidney (106), but confirmation of this is necessary.

## 4. Kininases

Kinin-inactivating enzymes are designated kininases. BK infused into the renal artery is not detected in the urine. This implies the abundant presence of kininases in the nephrons. They are distributed mainly in two parts of the nephron: the proximal tubules and the medullary CD.

The micropuncture technique revealed that almost all [ $^3\text{H}$ ]BK injected into the proximal tubules is destroyed in the tubules (107). Angiotensin-converting enzyme (ACE) also inactivates BK and is designated kininase II, which is concentrated in the proximal tubules along the brush-border membrane of the cells or in the  $\text{S}_3$  proximal tubule segments (101, 108, 109). A microdissection technique performed in the individual nephrons revealed that kininase activity is present not only in the proximal tubules, but also in the medullary CD (108).

We identified carboxypeptidase Y-like exopeptidase (CPY) and neutral endopeptidase (NEP) as major kininases in rat urine (110). Carboxypeptidase Y was originally found in yeast. This enzyme activity in rat urine was identified by means of the inhibitor spectrum and an antibody against a peptide fragment, but the overall structure could not be determined because of the small quantity of rat urine, so that it was tentatively designated CPY.

Accordingly, the degradation pathway of BK in rat urine is completely different from that in rat or human plasma (111, 112), as Fig. 5 shows. In the plasma, the major metabolite of BK is BK (1–5), or Arg-Pro-Pro-Gly-Phe, which was not detected in rat urine. The major metabolite of BK in rat urine is Arg-Pro-Pro-Gly-Phe-Ser, or BK (1–6) (112). The same was true in human urine (113).

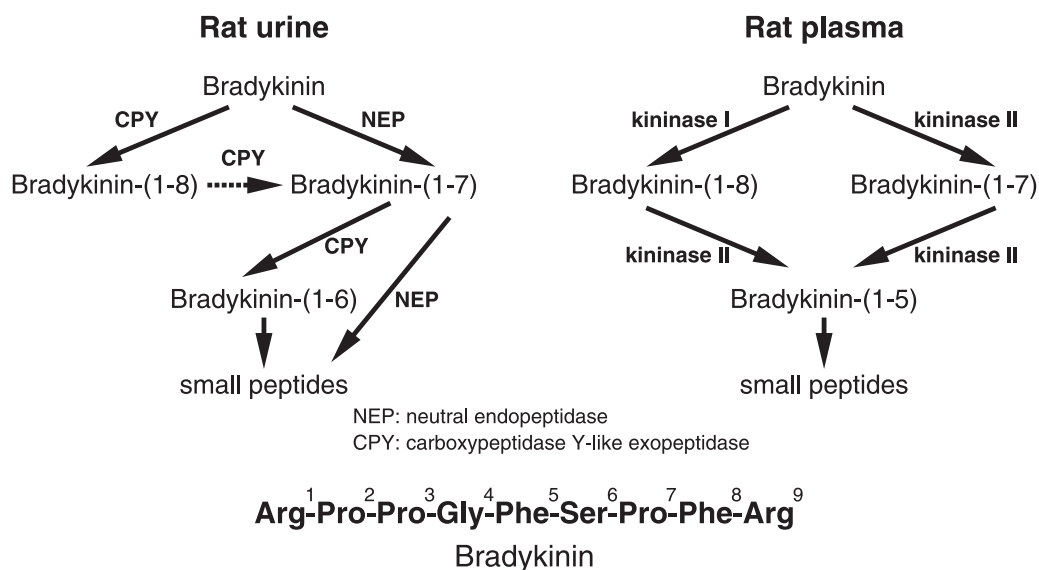
ACE inhibitors do not inhibit CPY, but ebelactone B, isolated from culture medium of *Actinomyces*, selectively inhibits the activity of this enzyme in rat urine without inhibiting plasma kininases activity (114). Treatment of anesthetized rats with ebelactone B during

infusion of physiological saline markedly exerts diuretic and natriuretic actions and causes increases in the kinin levels in the urine (114), indicating that CPY plays an active role in vivo by destroying kinin in the renal tubules. The other inhibitor, poststatin, isolated from a fermentation broth of *Streptomyces viridochromogenes*, also completely inhibits the degradation of BK by rat urine without affecting that in rat plasma (111), indicating that poststatin may inhibit both CPY and NEP.

NEP was reported to be present in the outer surface of the brush-border plasma membrane of the proximal tubules (115) and to a lesser extent in the vesicular organelles, being found both in the apical cytoplasm and on the basal infoldings of the proximal tubule cells (116). Urinary NEP was reported to contribute more than half of the renal kininase activity in humans (117). However, the degradation rate of BK in human urine is dependent on its pH (113). The inhibitory effect of an NEP inhibitor, phosphoramidon, becomes obvious at neutral pH, while the ebelactone B, a CPY inhibitor, inhibits the activity at both neutral pH and acidic pH.

### 5. A tissue kallikrein inhibitor

Kallistatin, a natural tissue kallikrein inhibitor, is biosynthesized mainly in the liver, but also, at lower expression levels, in the kidney (118). The mRNA of kallikrein-binding-protein (KBP), an analogue of human kallistatin, was detected most abundantly in the inner medullary CD, with only small amounts (about 1/10) in the outer medullary CD, proximal convoluted tubules, and glomeruli; no signals were found in the CNT or the cortical CD (119).



**Fig. 5.** Pathways of bradykinin (BK) degradation by rat urine and plasma. BK(1-n) indicated BK degradation products with n amino acids from the N-terminal. From ref. 162 with permission.

Renal kallikrein, its inhibitor (kallistatin), LMW kininogen, two tubular-specific kininases (CPY and NEP), and kinin receptors are entire components of the renal kallikrein-kinin system. Surprisingly, all these components are localized along the renal distal tubules, particularly from the CNT to the CD. Thus, once the renal kallikrein is secreted, it releases kinin from LMW kininogen and is efficiently inhibited by kallistatin after kinin has been bound to its receptor in the CD. The released kinin is then immediately inactivated by CPY and NEP.

## 6. Stimuli for kallikrein secretion in the kidney

### 1) Sodium

Sodium restriction accelerates the excretion of renal kallikrein. In normal human subjects, intravenous water loading during prolonged sodium restriction produced a significant increase in kallikrein excretion, but not during the period of normal sodium intake (120). A low dietary sodium intake or sodium restriction has consistently been observed to increase urinary kallikrein excretion in humans (120–123) and in rats (124, 125). In micro-dissected segments of rabbit nephrons, low sodium intake markedly increases the levels of both active and inactive kallikreins in the granular portion of the distal convoluted tubules and in the cortical CD (or CNT) without altering either the distribution profile or the ratio of active- to total-kallikrein in the nephron or the urine. Thus it is plausible to consider that restriction of salt intake increases the urinary excretion of kallikrein.

### 2) Sodium-retaining steroid hormones

Prolonged sodium deprivation causes aldosterone release through activation of the renin-angiotensin system. Thus, the increase in kallikrein excretion may occur together with the release of this hormone. In fact, a large accumulation of data indicates a positive correlation between the activity of sodium-retaining steroid hormone and the renal kallikrein-kinin system. Urinary excretion of kallikrein is increased 1) in patients with primary aldosteronism (48), 2) in normal volunteers or patients with essential hypertension on a diet of low sodium or high potassium (121), 3) after treatment with 9 $\alpha$ -fluorohydrocortisone (126), and 4) in Bartter's syndrome (hypertrophy and hyperplasia of the juxtaglomerular cells, producing hypokalemic alkalosis and hyperaldosteronism) (127). In addition, treatment of patients affected by primary aldosteronism and treatment of normal volunteers with spironolactone, a selective antagonist of aldosterone, markedly reduced urinary kallikrein excretion (121, 128). The removal of aldosterone-producing tumors also reversed the

increased excretion of urinary kallikrein (129).

It is clear that the administration of aldosterone enhances reabsorption of sodium ion and increases the urinary excretion of both potassium and hydrogen ions, and hypokalemia and alkalosis are induced. Accordingly, it is possible that increased plasma or intraluminal concentrations of potassium induced by aldosterone may accelerate kallikrein excretion.

### 3) Potassium

An electron-microscopic study (130) revealed that a high-potassium diet produced hypertrophy and hyperplasia of the kallikrein-containing cells, including hypertrophy of the components of the Golgi complex and of the rough endoplasmic reticulum, and a large number of secretory-type vesicles containing kallikrein. The results of this study suggest that a high-potassium diet increases the synthesis and secretion of kallikrein.

Intravenous infusion of anesthetized rats with high-potassium saline (67.5 mM KCl + 67.5 mM NaCl) over 90 min gradually increased the urine volume (by 47% in total) and the urinary excretion of Na<sup>+</sup> (by 32% in total) in parallel with an increase in the excretion of K<sup>+</sup> and Cl<sup>-</sup> (131). Urinary kallikrein showed a rapid increase of 49% within 60 min after the start of the K<sup>+</sup> infusion. The diuresis and natriuresis were almost completely suppressed by a BK B<sub>2</sub> antagonist, FR173657, indicating that diuresis and natriuresis by high-potassium infusion are attributable to renal kallikrein release.

The excretion of the renal kallikrein by intravenous infusion of high-potassium saline (K<sup>+</sup> 75 mM + Na<sup>+</sup> 75 mM) was rapid and was augmented in rats within 15 to 30 min, where infusion of physiological solution (150 mM NaCl) at the same speed did not significantly increase the kallikrein secretion (132). The rapid release could not be explained by aldosterone release, since the aldosterone release by intravenous infusion of potassium takes at least 1 h in humans (133). This rapid increase was further confirmed by an *in vitro* experiment, in which sliced cortex isolated from rat kidney was superfused with an isotonic solution containing more than 20 mM KCl (134). The addition of a physiological concentration (4 mM) of potassium into the superfusion fluid did not affect the release of renal kallikrein.

### 4) ATP-sensitive potassium channel blockers

The rapid release of renal kallikrein by potassium was thought to be mediated by the inhibition of the potassium channels of the CNT cells. Glibenclamide, an insulin releaser of the pancreas  $\beta$  cells, is an ATP-sensitive potassium (K<sub>ATP</sub>) channel blocker. Intravenous injection of glibenclamide (1 to 30 mg/kg) during physiological saline infusion in anesthetized rats causes a dose-

dependent increase in urinary kallikrein (132). Administration of another  $K_{ATP}$  channel blocker, PNU-37883A (135), also brought about increased urinary excretion of kallikrein together with increases in urine volume and sodium excretion (132). PNU-37883A has no additive effect on the potassium-induced increase in renal kallikrein secretion, suggesting that the site and the mode of action of potassium also may be the same as those of the  $K_{ATP}$  channel blockers, namely inhibition of potassium efflux and depolarization of the CNT cells. The release of renal kallikrein by glibenclamide (0.1 to 1  $\mu$ M) and PNU-37883A (10 to 100 nM) was also observed in the sliced rat kidney cortex in a concentration-dependent manner (134). PNU-37883A is selective to the CNT cells and 10 times more potent than glibenclamide, so that the  $K_{ATP}$  channels in the CNT cells may be slightly different from the  $\beta$  cells of the pancreas. Barium chloride, which inhibits intracellular potassium efflux by blocking the channel, also significantly increased the kallikrein release.

The apical low-conductance  $K^+$  channel, which is critical for  $K^+$  secretion into the lumen, is a member of the ATP-sensitive channel and is blocked by  $Ba^{2+}$  (136). Thus, it is likely that renal kallikrein secretion from the CNT cells is linked to inhibition of this low-conductance  $K^+$  channel that maintains the membrane potential of the tubular cell. Changes in the membrane voltage of the CNT cells may be one of the factors responsible for kallikrein secretion, since high potassium concentrations and administration of  $BaCl_2$  in the tubular lumen caused depolarization of the cell membrane voltage (137). The principal cells of the cortical CD, probably CNT cells, represent the only site where potassium is secreted in the nephron (136).

It is important that potassium or the  $K_{ATP}$  channel blockers can release kallikrein from the CNT cells, since the salt sensitivity of the BP is considerably attenuated by a high potassium intake.

## 7. Roles of the renal kallikrein-kinin system in the distal tubules

Many reviews on the role of the urinary kallikrein-kinin system, particularly in relation to hypertension, have been published (138–141).

The actions of BK on the renal functions have also been discussed in detail in a review article (10). Its major actions will be discussed in the following section.

### 1) Vasodilating action of BK in the kidney

Intravenous or intra-arterial administration of BK or kallidin induced renal arteriolar vasodilatation in normal human subjects and in anesthetized dogs or other animals. The role of endogenous kinins in the renal

vasodilatation has been discussed long and frequently, in particular under administration of inhibitors of ACE, which also degrades BK.

According to the study (142), in which optical fibers were implanted into the renal cortex or the renal medulla in rats, infusion of BK (0.1  $\mu$ g/min) into the renal medullary interstitium increased renal papillary blood flow (to 117% of the control) without altering cortical blood flow or BP in anesthetized rats.

The increased papillary blood flow was accompanied by a twofold increase in urine flow, sodium excretion, and fractional sodium excretion. Increases in papillary blood flow by BK may be related to the release of prostaglandins and NO.

It is possible that renal kallikrein, excreted into the basolateral side of the CNT cells, may generate kinins and increase the papillary blood flow in the kidney.

### 2) Diuresis and natriuresis by BK

Injection of kinins intravenously or into the renal artery induces diuresis and natriuresis (143, 144). It was proposed that the natriuretic effect of kinins may be attributed either to the inhibition of sodium reabsorption in the distal part of the nephron or to a change in deep nephron reabsorption due to the change in the blood flow.

Endogenous kinins generated in the tubular lumens of the CD act on BK  $B_2$  receptors distributed on the surface of the CD cells, inhibit sodium reabsorption and increase in urine flow and sodium excretion. Enteral administration of ebelactone B, a selective inhibitor of CPY (a major kininase in urine), induced diuresis and natriuresis (114), indicating that the inhibition of kinin degradation in the tubular lumens induced diuresis and natriuresis.

In isolated, perfused rat cortical CD, BK inhibited net sodium absorption and net chloride absorption without affecting net potassium transport, bicarbonate flux, or the transmembrane potential difference (101, 145).

### 3) Evidences that kinins act on the epithelial cells in the tubular lumens of the distal nephrons

Some of the typical diuretics, such as acetazolamide, which acts on the proximal tubules; furosemide and bumetamide, which act on the ascending limb of the loop of Henle; and thiazide, which acts on the distal tubules, all accelerate the excretion of urinary kallikrein. Accompanied excretion of renal kallikrein further elevated the urinary levels of sodium, potassium, and water induced by these diuretics.

Intravenous administration of furosemide to anesthetized rats during infusion of physiological saline, dose-dependently increased both the urinary excretion of renal kallikrein and the urine volume in the first

15 min (146). The excretion of sodium, chloride, and potassium was also increased. Pretreatment with a BK B<sub>2</sub> receptor antagonist, FR173657 (100 mg/kg), significantly reduced the peak values of the furosemide-induced natriuresis by nearly 50% without affecting potassium excretion. This implies that sodium, potassium, and chloride, which are delivered to the distal tubules from the loop of Henle, stimulate renal kallikrein excretion and add further excretion of sodium and chloride. Diuresis and natriuresis by administration of ebelactone B, a selective inhibitor of CPY (114), also provide clear evidence that endogenous kinins act on the distal tubules.

## **VI. Urinary kallikrein in hypertensives, African-Americans, elderly subjects, and potassium intake**

### **1. Urinary kallikrein in hypertensives**

As discussed above, both white and black subjects with essential hypertension excrete less urinary kallikrein than do their respective controls, but the excretion levels in hypertensive blacks are lower than those in normotensive whites (49). High potassium intake increased the excretion of urinary kallikrein and aldosterone according to the dietary potassium levels in both hypertensive and normotensive subjects. However, some studies reported that white patients with uncomplicated essential hypertension showed normal kallikrein excretion rates, normal plasma renin activity, and normal aldosterone levels (147) and that only hypertensives over 40 years of age excreted significantly less urinary kallikrein (148). It was reported that the population with low kallikrein excretion may represent 20% of hypertensive patients (149).

A low kallikrein excretion rate may be accompanied by low plasma renin activity (150). Among Japanese patients with low-renin hypertension, there are significant reductions in both active urinary kallikrein and kinin excretion together with increased levels of a kallikrein-inhibiting substance and kininase in the urine accompanied by reduced levels of kininogen (151).

Urinary kallikrein excretion was decreased in hypertensive patients with mild renal insufficiency (152) and markedly decreased in those with reduced glomerular filtration rates, as in those with hypertension (153). Renal parenchymal diseases accompanied by hypertension, such as chronic glomerulonephritis, are associated with diminished urinary kallikrein activity (152). Therefore, reduced levels of urinary kallikrein in hypertension should be distinguished from those due to impaired renal function in the elderly subjects.

### **2. Urinary kallikrein in African-Americans**

During unrestricted sodium intake, urinary kallikrein activity was greater in white normotensives than in white hypertensives or black normotensives, each of whom had a constant level of potassium intake. It has been claimed that there was no difference in urinary kallikrein between white and black hypertensives or between black normotensives and black hypertensives (120). All groups had greater urinary kallikrein activity on low sodium than on unrestricted sodium intake, but the increase in black hypertensives was small, and they excreted significantly less kallikrein than the other groups on the low sodium diet. Urinary kallikrein activity was reported to be correlated with renal blood flow in all groups except the black normotensives on a low sodium intake (120).

Epidemiological surveys also indicate that the urinary kallikrein concentration in random urine is significantly lower in black children than in white children and is positively correlated with the urinary creatinine and urinary potassium concentrations, but inversely related to the urinary sodium level (154). Families with the lowest kallikrein concentrations tended to have higher BP than did those with the highest concentrations, although the positive correlation was weak and subject to many variables (155). The significant inverse relationship between the urinary kallikrein levels divided by creatinine concentration and the BP in both white and black children was confirmed after 4 years of observation (155). The familial aggregations of BP, BP rank, and concentration of kallikrein in random urine were relatively stable in children over an 8-year period of observation (156).

It might be related to the reduced urinary kallikrein activity that black race and female gender were both associated with significantly greater systolic and diastolic BP responses to hydrochlorothiazide (157).

### **3. Potassium and urinary kallikrein**

High potassium intake increased the excretion of urinary kallikrein and aldosterone according to the dietary potassium levels in both hypertensive and normotensive subjects (158). The urinary kallikrein levels in hypertensives were always higher than in normotensives, but no statistically significant differences were observed in urinary kallikrein excretion between them. In both groups, the increase in white subjects is significantly higher than that in black subjects.

A randomized, crossover, double-blind study conducted for 4 days in 22 patients of at least 60 years of age revealed a decrease in SBP during potassium chloride ingestion (120 mmol/day) (159). More sodium,



potassium, and aldosterone were excreted during the daytime, while urinary kallikrein was excreted at a fixed rate throughout both day and night (160).

## VII. Anti-hypertensive effects of the agents related to the renal kallikrein-kinin system

The above reports indicate that the BP of African-Americans appears to be sensitive to a high salt intake and that they excrete less urinary kallikrein.

Mutant kininogen-deficient BN-Ka rats are quite sensitive to a salt intake and their SBP increases, as discussed above.

Thus, the normally acting renal kallikrein-kinin system plays the role of a safety valve for excess sodium intake (10, 161, 162) and the reduced function of the renal kallikrein-kinin system may lead to salt-sensitive hypertension after a high salt intake. Accordingly, enhancement of the natural activity of the renal kallikrein-kinin system must suppress the salt-sensitive hypertension or other hypertensions.

As mentioned above, renal kallikrein secretion from the CNT cells is enhanced by  $K_{ATP}$  channel blockers. Oral administration of glibenclamide (a  $K_{ATP}$  channel blocker, 30 mg/kg, twice a day) and a kidney-selective  $K_{ATP}$  channel blocker, U18177 (30 mg/kg, twice a day), suppressed the SBP rise in SD rats that received 8% NaCl in their diet and induced natriuresis and an increase in urinary kallikrein secretion (a manuscript in preparation). These effects were nullified by co-administration of a BK  $B_2$ -receptor antagonist, FR17365, indicating that  $K_{ATP}$  channel blockers induce diuresis and natriuresis by releasing renal kallikrein.

Ebelactone B is a renal CPY kininase inhibitor. In DOCA-salt hypertension, it completely prevented the hypertension, whereas a ACE inhibitor, lisinopril, had no effect on the development of hypertension (163, 164). Poststatin, another CPY kininase inhibitor, also inhibited the SBP rise in this model (164). An NEP inhibitor, BP102, also suppressed the SBP rise, but its effect was weaker than that of ebelactone B. The sodium levels in the serum, CSF, and erythrocytes in the DOCA-salt rats were all normalized by ebelactone B or BP102 (163, 165). In addition, inhibition of the CPY kininase activity by *in vivo* transfer of antisense oligonucleotide against CPY kininase also inhibited the SBP rise although only for a short time (166).

In SHR, supplementation with 1% KCl increased the urinary excretion of kallikrein and attenuated the BP by 18 mmHg after 5 weeks and also reduced renin activity (167).

Even in normotensive subjects and patients with WHO stage I essential hypertension, oral potassium

loading stimulated the release of urinary kallikrein. However, patients with stage II essential hypertension excreted a lower basal level of urinary kallikrein, and oral potassium did not increase this urinary kallikrein excretion (168).

When these findings are taken together, it is reasonable to conclude that less excretion of urinary kallikrein causes salt-sensitivity of the SBP, whereas enhancement of the renal kallikrein-kinin system due both to the increased secretion of the renal kallikrein by potassium or  $K_{ATP}$  channel blockers and to the increase in the urinary kinin level caused by renal kininase inhibitors may suppress the salt-induced hypertension.

## VIII. Conclusions

1. Approximately one-third of the normotensive subjects are reported to be salt-sensitive. African-Americans, hypertensives, and elderly subjects show particularly high sensitivity in their BP to salt loading.

2. Potassium intake induces strong suppression of the salt sensitivity of the BP.

3. Many factors have been proposed to link a high salt intake to the BP rise, but none of them provides a satisfactory explanation on its own.

4. We propose that the reduced function of the kallikrein-kinin system in the kidney may link a high salt intake to the BP elevation. The reasons are as follows: a) all components of this system are localized from the CNT cells (kallikrein) along the CD of the distal nephron. b) BK-degrading enzymes (kininases: CPY and neutral peptidase) in the nephron are quite independent from the surrounding plasma kininases. These kininases are inhibited by, for example, ebelactone B and poststatin. c) Renal kallikrein is secreted by potassium or  $K_{ATP}$  channel blockers. d) The renal kallikrein-kinin system exerts its role as a safety valve for the excess sodium intake since kininogen-deficient mutant BN-Ka rats, which cannot generate urinary kinin, are much more sensitive to lower concentrations of sodium intake than normal rats. e) Human normotensive subjects, who show lower urinary excretion of kallikrein, are salt-sensitive, and salt-sensitive hypertensive patients secrete lower levels of urinary kallikrein.

5. Both acceleration of renal kallikrein secretion and urinary kininase inhibition suppress the BP rise induced by a high salt intake.

## IX. Perspective

1. On the basis of these results, it should be investigated whether a salt-sensitive group in normotensives and hypertensives really shows reduced levels of urinary



kallikrein. In these clinical trials, potassium intake should be strictly controlled. If it is verified in large-scale of trials, then we may acquire an excellent marker for detection of salt-sensitive subjects with reduced levels of urinary kallikrein. The clinical entity of salt-sensitive hypertension will be clearly demarcated, so that severe salt-restriction will be limited to the certain subjects.

2. Pharmaceutical development of a) better  $K_{ATP}$  channel blockers specific to the CNT cells, and b) selective inhibitors of renal kininase, such as ebelactone B and poststatin, will, without doubt, open a new era in the field of the anti-hypertensive drugs and the number of patients with high BP will be substantially reduced by such novel anti-hypertensive drugs.

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