Crucial suppressive role of renal kallikrein-kinin system in development of salt-sensitive hypertension

MAKOTO KATORI*, MASATAKA MAJIMA and IZUMI HAYASHI

Department of Pharmacology, Kitasato University School of Medicine, Sagamihara, Kanagawa 228-8555, Japan

Tissue kallikrein and low molecular weight kininogen are localized in the particular cells of the connecting tubules, indicating that kinin is immediately generated in the lumina of the lower nephrons. The role of the renal kallikreinkinin system was studied using mutant kininogen-deficient Brown NorwayKatholiek (BN-Ka) rats, and compared with that in normal BN-Kitasato rats of the same strain. Mutant BN-Ka rats showed no visible changes, but they were very sensitive to excess sodium ingestion and to the tendency of sodium to accumulate in the body by aldosterone released by angiotensin II, so that sodium was accumulated in erythrocytes and cerebrospinal fluid in BN-Ka rats and hypertension was induced. After four days infusion of 0.3 M NaCl solution to conscious and unrestrained mutant BN-Ka rats, the sensitivity of the vascular smooth muscle to norepinephrine and angiotensin II increased 30-fold and 10-fold, respectively. Bradykinin was degraded by neutral endopeptidase (NEP) and carboxypeptidase Y-like exopeptidase (CPY) in rat and human urine. Daily oral administration of a selective inhibitor of CPY, ebelactone B, or that of NEP, BP1O2, prevented development of deoxycorticosterone acetate-salt hypertension in Sprague-Dawley rats. These results indicate that: 1) the renal kallikrein-kinin system allows excretion of excess sodium in the body, 2) decreased sodium excretion due to reduced excretion of urinary kallikrein in patients with essential hypertension or in genetically hypertensive rats may cause hypertension, and 3) urine kininase inhibitors such as ebelactone B may emerge as a new anti-hypertensive drug.

Key terms: *Brown Norway-Katholiek rats, Brown Norway-Kitasato rats, ebelactone B, DOCA-salt hypertension, renal kallikrein.*

DEDICATION

It is almost thirty years, since the International Kinin Symposium was held in 1969 in Fiezole, Italy, and so it has perhaps now faded from most memories. But it was the occasion of the author's first meeting with Prof Héctor Croxatto. In the years both before and since that Symposium, he has made great efforts and great contributions, particularly in the field of renal kallikrein with its purification and study in its relation to hypertension. Some of the work currently being carried out in our laboratories using kininogen-deficient mutant rats treads in his footsteps and is indebted to him.

We would like to dedicate this mini-review to Prof Héctor Croxatto on both his 90th birthday and his 65 years of continuous contribution to the relation between the kinin field and cardiovascular and renal functions in health and diseases.

* **Correspondence to:** Prof Makoto Katori, MD, PhD, Higashi 1-14-3, Shibuya-ku, Tokyo 150-0011, Japan. Fax: (81-3) 5466-4663. Phone: (81-3) 5466-4661. E-mail: hy3m-ktr@asahi-net.or.jp

Biol Res 31: 143-149 (1998)

INTRODUCTION

Recent histochemical and other studies have revealed that renal kallikrein is localized in cells in the connecting tubules of the nephron and that low molecular weight (LMW) kininogen is found in the principal cells in the connecting tubules (Figueroa *et al*, 1988). These sites are adjacent, and once renal kallikrein and LMW kininogen are secreted, kinin is promptly generated in the tubules. Kallistatin, a tissue kallikrein inhibitor, is also secreted in the collecting ducts of the inner medulla (Chen *et al*, 1995). Bradykinin B_2 receptors are distributed along the collecting ducts (Figueroa *et al*, 1995). These observations clearly suggest that the renal kallikrein-kinin system may have some roles to play in the kidney, but its precise roles have not yet been clarified.

We have had an opportunity to study the roles of the renal kallikrein-kinin system using mutant kininogen-deficient Brown Norway-Katholiek (BN-Ka) rats or natural knockout rats (Katori & Majima, 1996). Here, we introduce some recent findings on the roles of the renal kallikrein-kinin system in rats in relation to the development of hypertension. The detailed results have been published in review articles (Majima & Katori, 1995; Katori & Majima, 1996, 1998).

KININOGEN-DEFICIENT BROWN-NORWAY KATHOLIEK RATS

Mutant BN-Ka rats have the ability to produce high molecular weight (HMW) and LMW kininogens in the liver, but the point mutation of alanine^{163} to threonine in the common chain of the kininogens prevented a split into active forms (Hayashi *et al*, 1993), so that negligible levels of HMW and LMW kininogens were found in the blood stream and practically no kinin was detected in the urine, unlike the situation in normal BN-Kitasato (BN-Ki) rats of the same strain (Fig 1) (Majima *et al*, 1991). By using these mutant rats, we were able to exclude the contribution of the kallikrein-kinin system in the living rats. Interestingly, there was no visible alteration in these mutant BN-Ka rats. The systolic blood pressure increase that advances from the weaning stage was completely the same as that in normal BN-Ki rats, as long as they were fed with low levels (0.3%) of sodium chloride (Majima *et al*, 1991).

ACCUMULATION OF SODIUM IN BN-Ka RATS AFTER INGESTION OF EXCESS SODIUM

Mutant BN-Ka rats were very sensitive to excess ingestion of sodium chloride (Majima *et al*, 1993). Administration of 2% NaCl in the diet did not increase the

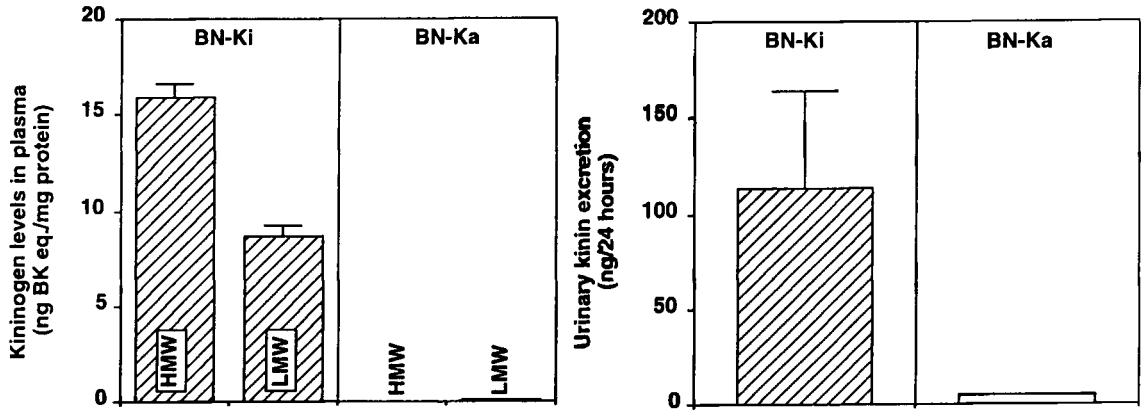

Fig 1. Kininogen levels in plasma (left panel) and urinary kinin excretion (right panel) in normal Brown Norway Kitasato (BN-Ki) rats and mutant BN-Katholiek rats (BN-Ka). Bars, means + SEM's of 4 rats. BK eq, bradykinin equivalent; HMW, high molecular weight; LMW, low molecular weight. (Reproduced from Majima *et al*, 1993, with permission)

systolic blood pressure in normal BN-Ki rats, whereas in mutant BN-Ka rats, the same treatment caused the blood pressure to rise to 167 ± 4 mm Hg within two weeks. Mutant BN-Ka rats drunk more water and excreted less urine and urinary sodium, than the normal BN-Ki rats did (Majima *et al*, 1993). After two weeks of ingestion of 2% NaCl, the sodium level in the erythrocytes in mutant BN-Ka rats was significantly increased, indicating that mutant BN-Ka rats, which failed to generate kinin in the urine, excreted less sodium while accumulating it in the cells. Supplementation of LMW kininogen infused subcutaneously with a mini-osmotic pump during 2% NaCl ingestion restored the kinin level in urine and the systemic pressure and increased the urinary volume and sodium excretion, indicating a direct relationship between blood pressure increase, sodium excretion and kininogen deficiency (Majima *et al*, 1993).

Continuous infusion of 6 ml/kg/hr of 0.3 M NaCl into the abdominal aorta of conscious and unrestrained mutant BN-Ka rats increased the mean arterial pressure, measured through an indwelling catheter, from 106 ± 3 mm Hg to nearly 127 ± 3 mm Hg within 24 hours (Majima *et al*, 1995a). Accumulation of sodium was observed a short time after infusion commenced. Sodium was accumulated in the erythrocytes and cerebrospinal fluid (CSF). The mean arterial pressure of mutant BN-Ka rats did not change during the arterial infusion of 6 ml/kg/hr of physiological saline (0.15 M NaCl) solution. Normal BN-Ki rats did not show increases in the mean arterial pressure or the sodium level in the erythrocytes and the CSF after administration of either 0.15 and 0.3 M NaCl solutions. These observations indicated that arterial infusion of a large volume (6 ml/kg/h) did not itself increase the blood pressure, and only concentrations of NaCl higher than that of physiological saline solution caused an increase in the systemic pressure together with sodium accumulation in mutant BN-Ka rats.

Kininogen-deficient BN-Ka rats accumulated sodium in the body not only by being loaded with excess sodium, but also by releasing aldosterone (Majima *et al*, 1994b). Continuous subcutaneous infusion of a non-pressor dose (20 μg/day/rat) of angiotensin II with a miniosmotic pump for two weeks to mutant BN-Ka rats resulted in an increase of the systolic pressure to 180 ± 8 mm Hg, together with an increased heart rate and accumulation of sodium in the erythrocytes and the CSF. The same treatment did not induce any change in these parameters in normal BN-Ki rats. Simultaneous treatment of mutant BN-Ka rats with spironolactone, an aldosterone antagonist, in the second week of angiotensin II infusion normalized the blood pressure, heart rate and erythrocyte and CSF sodium accumulation. Urinary aldosterone excretion was increased during angiotensin II infusion, but there was no difference in level between BN-Ka and BN-Ki rats. These findings clearly indicated that failure of kidney to generate kinin caused sodium accumulation in the cells and body fluid, once excess sodium was ingested or sodium accumulation occurred as a result of aldosterone release due to angiotensin II.

In summary, renal kallikrein does not take an active role in the excretion of sodium when the amount of sodium ingested is within physiological limits, but once sodium is ingested in excess or sodium begins to accumulate, then it takes action against the accumulated excess sodium and excrete it. It could be hypothesized that the renal kallikrein-kinin system works as a floodgate for excess sodium, as illustrated with a diagram in Figure 2 (Majima & Katori, 1995).

SITE OF ACTION OF KININ IN TUBULES FOR URINARY SODIUM EXCRETION

Kinin generated by renal kallikrein may act on vascular smooth muscle outside the renal tubules to accelerate sodium excretion, but there is evidence that the major site of action of kinin is the luminal side of the tubules.

The presence of kininase in urine has been reported (see Katori & Majima, 1996), but the degradation pathway of bradykinin

Biol Res 31: 143-149 (1998)

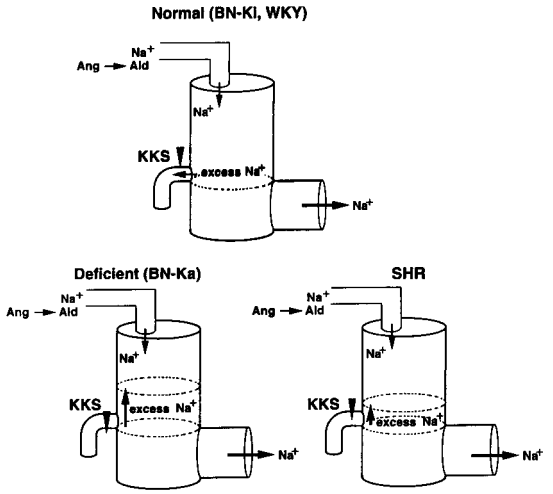

Fig 2. Role of the kallikrein-kinin system (KKS) in the kidney. BN-Ki, normal Brown Norway-Kitasato rats; BN-Ka, mutant Brown Norway-Katholiek rats; Ang, angiotensin II; Ald, aldosterone; SHR, spontaneously hypertensive rats. (Reproduced from Majima & Katori, 1995, with permission).

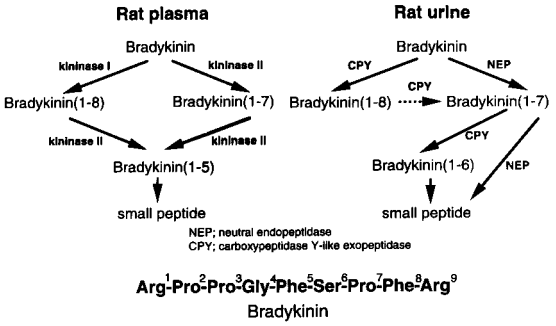

Fig 3. Pathway of bradykinin degradation by rat plasma and rat urine. Bradykinin (BK)-(I-n): BK degradation products with n amino acids from the N-terminal. (Reproduced from Katori *et al*, 1996, with permission).

in the urine was quite different from that in plasma (Kuribayashi *et al*, 1993). Major kininases in rat urine were neutral endopeptidase and carboxypeptidase Y-like exopeptidase (Kuribayashi *et al*, 1993), the latter of which was originally found as a carboxypeptidase in yeast. The same two peptidases were also present in human urine as major kininases. As shown in Figure 3, in human or rat plasma, bradykinin (BK) was degraded to BK (1-8) by kininase I (carboxypeptidase N) and to BK (I-7) by kininase II (angiotensin converting enzyme), and then to BK (I-5), again by kininase II. BK (1-5) is a rather stable BK metabolite that was detected in inflammation sites (Majima *et al*, 1996), even when BK could not be detected. In rat urine, BK (1-5) was not detected, as Figure 3 shows.

We found a selective inhibitor against carboxypeptidase Y-like exopeptidase, ebelactone B (Majima *et al*, 1994a), which does not inhibit BK degradation in the plasma, but degradation of BK in the urine was suppressed by about 50% by ebelactone B. Intraduodenal administration of ebelactone B induced a significant increase in the urinary kinin level, urine volume and urinary sodium, but not in urinary potassium. This increase was completely inhibited by the BK B_2 receptor antagonist, Hoe 140 (Majima *et al*, 1994a). These results clearly indicated that diuresis by BK occurs on the tubular side.

RELATION OF SODIUM ACCUMULATION TO DEVELOPMENT OF HYPERTENSION

Ingestion of excess sodium or infusion of a non-pressor dose of angiotensin II always increased the systolic blood pressure in kininogen-deficient mutant BN-Ka rats. This was accompanied with sodium accumulation in the erythrocytes and the CSF (Majima *et al*, 1993, 1994b).

Continuous infusion of 0.3 M NaCl solution into the abdominal aorta of conscious and unrestrained mutant BN-Ka rats for four days caused a shift to the left of the dose-response curve of the systemic blood pressure to intravenous norepinephrine (1-1000 pmol/kg) (Majima *et al*, 1995a). The sensitivity to norepinephrine was increased 30-fold and that to angiotensin II was 10-fold. This may be attributable to accumulation of sodium in the arterial smooth muscle, just as in erythrocytes. These observations suggest that the blood pressure can be increased without any increase in the concentrations of vasoactive substances in the blood stream, but merely by an increase in sensitivity of the vascular smooth muscle.

Furthermore, sodium accumulation in the CSF may increase the sympathetic tone, since continuous infusion of different

concentrations of sodium into the cisterna magna of rats increases the systemic blood pressure together with an increase in the sympathetic discharge (Sasaki *et al*, 1984). Increased sympathetic tone has been repeatedly reported in hypertension.

PREVENTION OF DOCA-SALT HYPERTENSION BY INFUSION OF EBELACTONE B

Deoxycorticosterone-acetate (DOCA)-salt hypertension was induced in Sprague-Dawley rats by uninephrectomy at 6 weeks of age and by weekly injection of DOCA (5 mg/kg, sc) and 1% NaCl in drinking water. As shown in Figure 4, the systolic blood pressure, measured by the tail cuff method, gradually increased up to 10 weeks of age. As is well known, prolonged administration of the angiotensin converting enzyme inhibitor, lisinopril, did not affect the increase in the systolic pressure in this model. In contrast, administration of ebelactone B completely prevented the increase in the systolic pressure (Yto *et al*, 1997). A selective inhibitor of neutral endopeptidase, EP102, also prevented the development of DOCA-salt hypertension, since major kininases of rat urine are carboxypeptidase Y-like exopeptidase and neutral endopeptidase (Kuribayashi *et al*, 1993). Administration of ebelactone B for one week also suppressed the increase in the systolic blood pressure in normal BN-Ki rats (Majima *et al*, 1995b). These results indicate that the renal kallikrein-kinin system plays an important suppressive role during the development of DOCA-salt hypertension.

In this DOCA-salt hypertension model, normal uninephrectomized BN-Ki rats 6 weeks of age treated with DOCA and salt excreted increasing amounts of urinary kallikrein until 10 weeks of age, when the amount began to decline (Katori *et al*, 1992). The systolic blood pressure increased gradually thereafter, reaching 180 ± 10 mm Hg at 17 weeks of age. Increased excretion of urinary kallikrein was accompanied with increase in urine volume and sodium excretion in normal BN-Ki rats. Kininogen-deficient BN-Ka rats excreted urinary kallikrein to the same degree, but this process

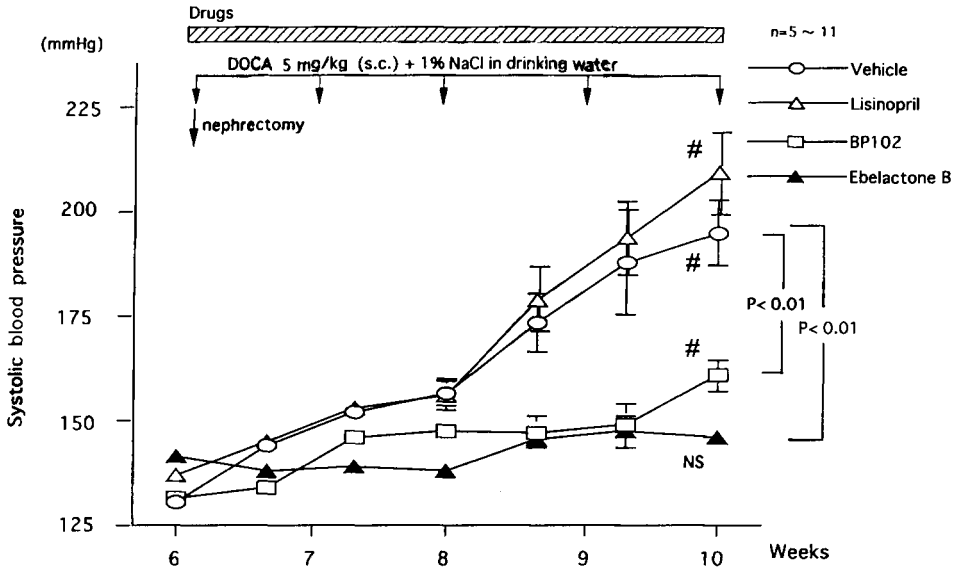

Fig 4. Prolonged administration of kininase inhibitors to deoxycorticosterone acetate (DOCA)-salt hypertension in Sprague Dawley rats. DOCA-salt hypertension induced by uninephrectomy at 6 weeks of age and by weekly injections of DOCA (5 mg/kg, sc) + 1% NaCl in drinking water. Systolic blood pressure (SBP) was measured using tail cuff. Daily oral administrations of lisinopril (5 mg/kg, twice a day), ebelactone B (5 mg/kg twice a day) or BP102 (30 mg/kg twice a day) made from first day of the DOCA-salt treatment. Symbols, means ± SEM's of 5-11 rats. Values at 10 weeks of age compared with own values at 6 weeks of age by Students's *t*-tests. # $p < 0.05$; NS, not significant. All values from each experimental group mutually compared by repeated ANOVA.

was not accompanied by increases in urine volume and urinary sodium excretion, and the systolic pressure reached a peak 2 weeks after the start of treatment (Majima *et al*, 1991). These results from kininogen-deficient BN-Ka rats clearly indicate that the renal kallikrein-kinin system has a crucial suppressive role in the development of DOCA-salt hypertension. Furthermore, the site of action of sodium secretion accelerated by kinin is on the luminal side and not basolateral side, since ebelactone B prevents the development of this hypertension.

It was reported that patients with essential hypertension (Margolius *et al*, 1971), Okamoto-Aoki's spontaneous hypertensive rats and other genetically hypertensive rats excrete reduced levels of urinary kallikrein. This was also reported by Prof Croxatto (Croxatto & San Martin, 1970; Croxatto *et al*, 1974, 1976). In genetically hypertensive rat models or hypertensive patients, the reduced levels of urinary kallikrein generate less kinin in the renal tubules, which may cause reduced excretion of sodium and induce sodium accumulation and hypertension, as shown in Figure 2. Thus, the administration of ebelactone B or BP102 caused an increase in the level of BK in the renal collecting ducts through inhibition of BK degradation, these types of agents offer a new strategy of antihypertensive treatment, at least for salt-sensitive hypertension.

SUMMARY

The renal kallikrein-kinin system acts to excrete sodium when excess amounts are ingested or when sodium is accumulated in the body by aldosterone release induced by angiotensin II. In DOCA-salt hypertension, increases in kinin levels in the renal tubules caused by the suppression of BK degradation by urinary kininase inhibitors such as ebelactone B prevents the development of the hypertension.

REFERENCES

CHEN LM, SONG Q, CHAO L, CHAO J (1995) Cellular localization of tissue kallikrein and kallistatin mRNAs in human kidney. Kidney Intl 48: 690-697

CROXATTO HR, SAN MARTIN M (1970) Kallikrein-like activity in urine of renal hypertensive rats. Experientia 26: 1216-1217

CROXATTO HR, ALBERTINI R, ROBLERO J, CORTHORN J (1974) Renal kallikrein (kininogenase activity) in hypertensive rats. Acta Physiol Latinoam 24: 439-442

CROXATTO HR, ALBERTINI R, ARRIAGADA R, ROBLERO J, ROJAS M, ROSAS R (1976) Renal urinary kallikrein in normotensive and hypertensive rats during enhanced excretion of water and electrolytes. Clin Sci 51 (suppl): 259-261

FIGUEROA CD, MacIVER AG, MacKENZIE JC, BHOOLA KD (1988) Localization of immunoreactive kininogen and tissue kallikrein in the human nephron. Histochemistry 89: 437-442

FIGUEROA CD, GONZALEZ CB, GRIGORIEV S, HAASEMANN M, JARNAGIN K, MÜLLER-ESTERL W (1995) Probing for the bradykinin B_2 receptor in rat kidney by anti-peptide and anti-ligand antibodies. J Histochem Cytochem 43: 137-148

HAYASHI I, HOSHIKO S, MANABE O, OH-ISHI S (1993) A point mutation of alanine^{163} to threonine is responsible for the detective secretion of high molecular weight kininogen by the liver of Brown Norway Katholiek rats. J Biol Chem 268: 17216-17224

ITO H, MAJIMA M, IZUMI T, KATORI M (1997) Complete inhibition of development of DOCA-salt hypertension by prolonged administration of urinary carboxypeptidase Y-like kininase inhibitors ebelactone B. Hypertension 30: P-83 (Abstract)

KATORI M, MAJIMA M (1996) Crucial role of the renal kallikrein-kinin system in development of hypertension and approaches to new drugs based on this relation. Jpn J Pharmacol 70: 95-128

KATORI M, MAJIMA M (1998) Preventive role of renal kallikrein-kinin system in early phase of hypertension and development of antihypertensive drugs. Adv Pharmacol 44: 147-224

KATORI M, MAJIMA M, MOHSIN SSJ, HANAZUKA M, MIZOGAMI S (1992) Essential role of kallikrein-kinin system in suppression of blood pressure rise during the development of hypertension induced by deoxycorticosterone acetate-salt in rats. Agents Actions 38 (suppl): 235-242

KURIBAYASHI Y, MAJIMA M, KATORI M, KATO H (1993) Major kininases in rat urine are neutral endopeptidase and carboxypeptidase Y-like exopeptidase. Biomed Res 14: 191-201

MAJIMA M, KATORI M (1995) Approaches to the development of novel antihypertensive drugs: Crucial role of the renal kallikrein-kinin system. Trends Pharmacol Sci 16: 239-246

MAJIMA M, KATORI M, HANAZUKA M, MIZOGAMI S, NAKANO T, NAKAO Y, MIKAMI R, URYU H, OKAMURA R, MOHSIN SSJ, OH-ISHI S (1991) Suppression of rat deoxycorticosterone acetate-salt hypertension by kallikrein-kinin system. Hypertension 17: 806-813

MAJIMA M, YOSHIDA O, MIHARA H, MUTO T, MIZOGAMI S, KURIBAYASHI Y, KATORI M, OH-ISHI S (1993) High sensitivity to salt in kininogen-deficient Brown Norway Katholiek rats. Hypertension 22: 705 -714

MAJIMA M, KURIBAYASHI Y, IKEDA Y, ADACHI K, KATO H, KATORI M, AOYAGI T (1994a) Diuretic and natriuretic effect of ebelactone B in anesthetized rats by inhibition of a urinary carboxypeptidase Y-like kininase. Jpn J Pharmacol 65: 79-82

MAJIMA M, MIZOGAMI S, KURIBAYASHI Y, KATORI M, OH-ISHI S (1994b) Hypertension

induced by a non-pressor dose of angiotensin II in kininogen-deficient rats. Hypertension 24: 111-119

MAJIMA M, ADACHI K, KURIBAYASHI Y, MIZOGAMI S, KATORI M (1995a) Increase in vascular sensitivity to angiotensin II and norepinephrine after four-day infusion of 0.3 M sodium chloride in conscious kininogen-deficient Brown Norway Katholiek rats. Jpn J Pharmacol 69: 149-158

MAJIMA M, IKEDA Y, KURIBAYASHI Y, MIZOGAMI S, KATORI M, AOYAGI T (1995b) Ebelactone B, an inhibitor of urinary carboxypeptidase Y-like kininase, prevents the development of deoxycorticosterone acetate-salt hypertension in rats. Eur J Pharmacol 284: 1-11

MAJIMA M, NISHIYAMA K, YAO K, OGINO M, OHNO T, SUNAHARA N, KATOH K, TAKEMACHI N, TAKEI Y, KATORI M (1996) Determination of bradykinin-(1-5) in the inflammatory exudate by a new ELISA as a reliable mediator for bradykinin generation. Inflamm Res 45: 416-423

MARGOLIUS HS, GELLER R, PISANO JJ, SJOERDSMA A (1971) Altered urinary kallikrein excretion in human hypertension. Lancet ii: 1063-1065

SASAKI S, TAKEDA K, OKAJIMA H, TAKAHASHI H, YOSHIMURA M, NAKAGAWA M, IJICHI H (1984) Pressor responses to intracranial injection of hypertonic NaCl in rats. J Cardiovasc Pharmacol 6: 349-364