Sodium Accumulation Induces Hypertension in Kininogen Deficient Brown Norway Katholiek Rats. Masataka Majima and Makoto Katori. Department of Pharmacology, Kitasato University School of Medicine, Sagamihara, Kanagawa 228, Japan.

Kininogen deficient Brown Norway Katholiek rats, which showed very low levels of plasma kininogens (high molecular weight kininogen, 0.1 ± 0.1 ng Bradykinin equivalent/mg protein; low molecular weight kininogen, 0.05 ± 0.01 ng Bradykinin equivalent/mg protein, $n=6$), excreted a much smaller amount of kinin (less than 4.8 ng/24 hours, $n=4$) in the urine than normal rats (high molecular weight kininogen, 15.4 ± 0.5 ng Bradykinin equivalent/mg protein; low molecular weight kininogen, 9.5 ± 0.3 ng Bradykinin equivalent/mg protein; urinary kinin, 102 ± 21 ng/24 hours, $n=4-6$) of the same strain (Brown Norway Kitasato rats, BN-Ki). This mutant BN-Ka rats can provide the important information to understand pathophysiological roles of the kallikrein-kinin system, when normal BN-Ki rats were used as a control animal.

The systolic blood pressure was determined by the tail-cuff methods in parallel with chronic direct blood pressure measurement. Heart rate was also recorded during blood pressure measurement. The volume of urine and water intake for 24 hours were determined using the metabolic cages. The sodium and potassium levels in urine and serum were determined by selective electrodes. Urinary kinin levels in the urine collected from both ureters were determined by the specific enzyme immunoassay after purification of kinin fraction with Sep-Pak C18 columns and high performance liquid chromatography. Urinary kallikrein activity was determined by the hydrolytic activity for the synthetic substrate, Pro-Phe-Arg-MCA. Jaffe's reaction was used for the determination of creatinine levels in serum and urine. The sodium concentration in erythrocytes were measured by the atomic absorption, after washing red blood cells with ice-chilled isotonic lithium chloride solutions. Cerebrospinal fluid was collected from the cisterna magna of ether-anesthetized rats by aspiration with 26G needle, and sodium levels were determined by the atomic absorption. Plasma renin activity was measured by the angiotensin I generating activity using angiotensin specific radio immunoassay. Urinary excretion of aldosterone was measured by specific radio immunoassay after extraction of aldosterone fraction from urine. Hypertension was introduced by continuous subcutaneous infusion of angiotensin II for 2 weeks using a micro-osmotic pump (Alza, 2002) implanted on the back of the rats. For another sets of animals, high sodium diets (2-8% NaCl) were prepared by addition of NaCl to the low NaCl (0.3% NaCl, powder form) diets. These diets were given to the rats ad libitum. Distilled water was given as a drinking water. For continuous administration of kininogen, bradykinin antagonist and aprotinin, another type of micro-osmotic pump (Alza, 2001) was implanted on the back of the rats.

The systolic blood pressure of 7-week-old kininogen-deficient BN-Ka rats (132 ± 2 mmHg, $n=7$) was not different from that in normal BN-Ki rats (129 ± 3 mmHg, $n=7$). Subcutaneous infusion of angiotensin II ($20 \mu\text{g/day/rat}$) from 7 weeks of age for 2 weeks by a micro-osmotic pump caused significant increases in blood pressure (181 ± 5 mmHg, $n=7$, 9 weeks old) in deficient BN-Ka rats, although the same treatment induce no blood pressure increase in normal BN-Ki rats (132 ± 3 mmHg, $n=6$). During this period, heart rate of deficient BN-Ka rats (480 ± 30 beats/min, 8 weeks old) was significantly higher than that in normal BN-Ki rats (350 ± 15 beats/min, 8 weeks old) with angiotensin II. The deficient BN-Ki rats tended to excrete less urinary sodium and showed significantly higher sodium levels in serum (143.2 ± 0.3 mmol/l), erythrocytes (5.1 ± 0.2 mmol/l) and cerebrospinal fluids (146.8 ± 2.3 mmol/l), compared with normal BN-Ki rats (serum, 141.5 ± 0.4 ; erythrocyte, 3.7

± 0.3 ; cerebrospinal fluid, 138.8 ± 1.9 mmol/l) at 8 weeks of age. Urinary excretions of aldosterone were increased with angiotensin II in both deficient BN-Ka and normal BN-Ki rats ($p < 0.05$). Spironolactone treatment (50 mg/kg/day) for 7 days in deficient BN-Ka rats reduced the increased blood pressure (181 ± 5 mmHg, $n=7$) and heart rate (500 ± 10 beats/min, $n=7$) to the levels (137 ± 3 mmHg, 380 ± 30 beats/min, $n=7$), which were the similar levels to the normal BN-Ki rats with angiotensin II. This spironolactone treatment resulted in the significant ($p < 0.05$) reduction in sodium levels in the erythrocytes and cerebrospinal fluids. Subcutaneous infusion (5 mg/kg/day) of bovine low molecular weight kininogen with an osmotic pump in angiotensin II-treated deficient BN-Ka rats induced the significant reduction in blood pressure ($p < 0.05$), heart rate ($p < 0.05$) and sodium levels in the erythrocytes ($p < 0.05$). By contrast, subcutaneous infusion (5 mg/kg/day) of a bradykinin antagonist, Hoe140 in angiotensin II-treated normal BN-Ki rats induced a significant hypertensive response ($p < 0.05$) in parallel with the significant increase in heart rate ($p < 0.05$) and sodium levels in the erythrocytes ($p < 0.01$). Hypertensive responses in anesthetized mutant BN-Ka rats to angiotensin II (10-100 pmol/kg, i.v.) administered as a single bolus were not different from those in normal BN-Ki rats.

Two-percent NaCl diets, given from 7 weeks of age for 4 weeks, also caused rapid increases in blood pressure (167 ± 4 mmHg, $n=12$, 9 weeks old) in deficient BN-Ka rats, although the same diets induced no blood pressure increase in normal BN-Ki rats. Higher (3-8%) concentration of NaCl in diets also resulted in the significant and marked increase in blood pressure in mutant BN-Ka rats, however, normal BN-Ki rats induced less but significant blood pressure increase in normal BN-Ki rats compared with rats receiving low NaCl (0.3%) diets. Urinary excretion of active-kallikrein and pro-kallikrein remained constant in both groups of rats throughout 2% NaCl loading. Higher (3-8%) concentration of NaCl in diets caused the reduction in urinary excretions of kallikrein in both mutant BN-Ka rats and normal BN-Ki rats. During 2% NaCl loading period, the deficient BN-Ka rats secreted significantly less urine (9 weeks old, $p < 0.05$) and less urinary sodium (11 weeks old, $p < 0.05$). Serum levels of sodium in deficient BN-Ka rats were significantly higher ($p < 0.05$) than in normal rats at 9 weeks of age. Intracellular concentrations of sodium in the erythrocytes of deficient BN-Ka rats were significantly higher ($p < 0.05$) than in normal BN-Ki rats throughout 2% NaCl loading. Subcutaneous infusion (5 mg/kg/day) of bovine low molecular weight kininogen with an osmotic pump in 2% NaCl-loaded deficient BN-Ka rats induced a significant reduction ($p < 0.01$) of blood pressure and increases ($p < 0.05$) in urine volume and urinary sodium and kinin levels. By contrast, subcutaneous infusion of Hoe140 (5 mg/kg/day), or of aprotinin (10000 KIU/kg/day) in NaCl-loaded normal rats induced a hypertensive response. This antagonist treatment reduced urine volume ($p < 0.05$) and urinary sodium ($p < 0.05$) significantly.

These results indicated that the lack of kinin generation due to the congenital deficiency in kininogens was related through sodium accumulation to the hypertensive response to a non-pressor dose of angiotensin II administration or NaCl loading in diets.