65) Simultaneous determination of vascular and plasma levels of angiotensins and kinins by using radioimmunoassay in combination with gradient reversed-phase HPLC. Kazuo Nakamura, Takeo Matsukura, Mari Takamiya, Yoshiyuki Nakamura and Keiji Nakamura. Department of Pharmacology, Nippon Roche Research Center, Kamakura 247.

Angiotensins (AGs) and kinins synthesized locally in the vasculature may play an important role in the control of vasomotor tone and blood pressure. Nevertheless, no systematic data on vascular levels of AG I, II, III, bradykinin (BK), kallidin (KL) and ACE activity have been available. For the purpose of correlating AGs and kinins levels in vessels with their plasma levels and vascular ACE activity in rats, a new procedure for simultaneous separation of these vascular or plasma peptides by HPLC has been optimized.

Age-matched male SHR and WKY at 19-21 weeks of age were used. The thoracic aorta and mesenteric artery were rapidly removed from ether-anesthetized rats and freed from connective tissues and blood. Vessels pooled from 10 rats in hot 2 M HOAc containing 0.1 M C_2H_5SH were boiled, homogenized and centrifuged. Blood was collected into polystyrene tube containing inhibitor cocktail (EDTA 2Na, o-phenanthroline, soybean trypsin inhibitor, aprotinin, pepstatin A, chymostatin and bacitracin) and immediately centrifuged. Plasma acidified by 1/8 N HCl was boiled. After the extraction of peptides by reversible adsorption to Sep-Pak C_{18} cartridge, the extracts were ultrafiltrated using Mol-Cut II and lyophilized. HPLC system (LC-6A type, Shimadzu) with μ -Bondapak C_{18} (10 μ m, Waters) was used for simultaneous separation of peptides. Stepwise-gradient (16% acetonitrile from 10 mM ammonium formate, pH 3.2) and thereafter linear-gradient (35% from 16%) were performed. Immunoreactive peaks from both biological samples and authentic peptides mixture had the identical retention times to absorption peak at 210 nm. Anti-AG II serum (raised in our laboratory), anti-AG I serum (Amersham) and anti-BK serum (Biosys) were used for RIA. Detection limit of each RIA was 1 pg/tube for AG I, II, III, and 3 pg/tube for BK, KL and met-KL. Standard curves for each peptide were constructed by simultaneous separation of mixed peptides in the same way as the biological samples. Bovine serum albumin (0.1%) was added to polyethylene tubes to prevent adsorption of peptides. When compared with the inhibitor cocktail containing pepstatin A, the inhibitor cocktail without pepstatin A increased plasma AG I by 20% without a change in AG II and III levels, suggesting active involvement of renin in the preparation step. When plasma containing the inhibitor cocktail was stored for 3 days in liquid N_2 , AG I levels increased 3-fold, and AG II and AG III levels slightly decreased and increased, respectively. Accordingly, we could stabilize AGs levels with immediate extraction from the changes of basal levels using the complete inhibitor cocktail. In adult SHR, there was no significant difference in plasma AG I, II, III and KL levels as compared with those of WKY. Plasma BK levels (like ACE activity) seemed to be lower in SHR (46.8 $\pm$ 18.0 pg/ml) than in WKY (110 $\pm$ 41.4 pg/ml). Acute treatment (4 hr) of SHR with cilazapril at the threshold hypotensive dose of 0.3 mg/kg p.o. significantly lowered plasma AG II, III and ACE activity, elevated AG I and tended to lower KL. Preliminary studies on SHR with two vascular preparations each pooled from 10 rats indicated higher levels of AG III in the mesenteric artery than in the thoracic aorta, and no detection of AG II, BK, KL and met-KL. In both vessels, cilazapril decreased AG III levels and ACE activity, and could quantify BK levels. A major kinin in both vessels might be BK.

In summary, major detectable vascular AG and kinin were AG III and BK. In contrast to lower ACE activity in the mesenteric artery than in the thoracic aorta, AG III levels were higher in the mesenteric artery. In SHR, plasma BK levels and ACE activity were lower. Cilazapril decreased plasma AG II, III, KL levels and ACE activity, and increased AG I levels in SHR. In both vessels, the treatment decreased AG III and increased BK levels.