30) Binding sites for atrial natriuretic peptide and angiotensin II in the cerebral microvessels of spontaneously hypertensive rats. Masa-aki Ibaragi, Masami Niwa, Tetsu Maeda, Kimihiro Yamashita and Masayori Ozaki. Department of Pharmacology 2, Nagasaki University School of Medicine, Nagasaki 852, Japan.

INTRODUCTION: Atrial natriuretic peptides (ANPs) are thought to play a role in the regulation of Na^+ influx through a Na^+ channel or $\text{Na}^+\text{-Cl}^-$ cotransporter by interacting specific, high affinity receptors, partly in collaboration with the angiotensin II (ANG) system. Evidence for the existence of specific ANP and ANG binding sites in the cerebral microvessels (MV) aids in elucidating the possible role of the peptides on the regulation of water and electrolyte fluxes across the blood-brain barrier (BBB). In the present study, an attempt was made to determine the participation of ANP and ANG binding sites of the cerebral MV in the impairment of blood-brain permeability occurring in hypertension of spontaneously hypertensive rats (SHR).

MATERIALS AND METHODS: Male 4- (young) and 20-week-old (adult) SHR and Wistar Kyoto rats (WKY) were decapitated, and the cerebral cortex was immediately removed. The MV were isolated by the method of Goldstein et al. (1975) with a modification by Kobayashi et al. (1981), and purity was assessed by morphological criteria. The MV composed of capillaries were homogenized in 50 mM Tris-HCl buffer containing 10 mM EDTA-2Na, and centrifuged at 50,000G. ^{125}I labeled α -rat ANP (1-28)[ANF-(99-126)] (^{125}I -rANP) and ^{125}I labeled [Ile⁵] ANG (^{125}I -ANG) were used to recognize receptor binding sites of ANP and ANG, respectively. The pellets were suspended in the assay buffer, and incubated with ^{125}I -rANP or ^{125}I -ANG in different concentrations ranging from 10 pM to 1 nM. Nonspecific binding was determined in the presence of 1 μM unlabeled rANP and ANG. Data on the binding kinetics were analyzed using a LIGAND computer program.

RESULTS: Scatchard analysis of the radiolabeled ligands binding to the cerebral MV of SHR and WKY indicated that the ANP and ANG binding sites were apparently single and of high affinity. In adult SHR, the number of ANP binding sites was substantially lower than in adult WKY (Bmax-SHR 37 fmol/mg protein, Bmax-WKY 147 fmol/mg protein, $p < 0.01$, one-way ANOVA), with no changes apparent in the K_a (SHR 92 pM, WKY 121 pM). Conversely, a significant increase in the Bmax of ANG binding sites was observed in adult SHR (SHR 6.40 fmol/mg protein, WKY 3.08 fmol/mg protein, $p < 0.05$), whereas no changes in the K_d were detected (SHR 663 pM, WKY 881 pM). There were no differences in the binding kinetics of ^{125}I -rANP and ^{125}I -ANG between young SHR and WKY.

DISCUSSION: Accumulating evidence suggests that water and electrolyte-permeability across the BBB is linked and related to the etiology of cerebral complications of hypertension. We found a reciprocal change in the number of specific ANP and ANG binding sites in the cerebral MV, a site of the BBB, of adult SHR, but not young SHR. As this impairment seems to result from hypertension, the possibility that ANP and ANG may play a role in the etiology of BBB dysfunction in hypertension by interacting with specific receptors has to be considered.