36) An unique characteristic and possible function of atrial natriuretic peptide receptor in the rat cerebral capillaries. Masa-aki Ibaragi, Masami Niwa and Masayori Ozaki. Department of Pharmacology 2, Nagasaki University School of Medicine, Nagasaki 852, Japan.

INTRODUCTION: We recently reported evidence for specific binding sites of α -rat atrial natriuretic peptide (rANP) in the cerebral capillaries, a candidate of physiologically active ANP receptor functionally related to water and electrolyte permeability across the blood-brain barrier (BBB). The amiloride-sensitive Na^+ transporter is thought to participate brain sodium uptake across the BBB (Betz, Fed. Proc. 45, 2050, 1986). As it has been suggested that ANP inhibits amiloride-sensitive Na^+ transport into the renal epithelial cell line, LLC-PK1, and ANP receptor in the bovine adrenal zona glomerulosa contains an amiloride-sensitive effector protein, we studied the interrelationship between ANP receptor and amiloride-sensitive Na^+ uptake into the cerebral capillary endothelium. Also, we obtained evidence that ANP receptor in the cerebral capillaries is possibly coupled with islet-activating protein (IAP)-sensitive G protein.

MATERIALS AND METHODS: Cerebral capillaries were isolated from the cortex of adult Wistar rats and ^{125}I -rANP binding studies were carried out, as previously reported (Niwa et al, Neurosci. Lett., 91, 89, 1988). Effects of GTP and GppNHp in the incubation medium on ^{125}I -rANP binding were studied using the capillaries pretreated with 100 ng/ml IAP at 4°C for 24 h. Amiloride-sensitive $^{22}\text{Na}^+$ uptake into capillaries was measured, in the presence of 1 mM ouabain, 1 mM furosemide and 2 mM LiCl, according to Betz (J. Neurochem. 41, 1150, 1983).

RESULTS AND DISCUSSION: GTP and GppNHp strongly inhibited ^{125}I -rANP binding to capillaries, with K_i values of 247 μM and 1 mM. When pretreated with IAP, the inhibition was significantly attenuated. The number (B_{max}), 130 fmol/mg protein of binding sites in the capillaries pretreated with IAP was decreased to that of 48 fmol/mg protein. One hundred μM of amiloride changed ^{125}I -rANP binding kinetics, 170 pM of K_d and 159 fmol/mg protein, to those of 31 pM and 88 fmol/mg protein, respectively. Amiloride-sensitive $^{22}\text{Na}^+$ uptake into the capillaries was selectively inhibited by 0.1 μM rANP. There were no effects of rANP on Na^+ uptake under the conditions inhibiting the furosemide-sensitive $\text{Na}^+\text{-Cl}^-$ co-transporter, Na^+/H^+ exchanger and Na^+ , K^+ -ATPase. The inhibition of rANP on the amiloride-sensitive Na^+ uptake was much less potent in capillaries isolated from SHRSP, as compared with those of WKY and SHR, at the age of 20-26 weeks. Multiple subtypes of ANP receptors have been postulated. The present observation made in the characteristics of specific ANP binding sites in the cerebral capillary endothelium led to the suggestion of existence of ANP receptor coupled with IAP-sensitive G protein. The possibility that the receptor operates Na^+ permeability across the BBB by interacting with the amiloride-sensitive effector would have to be considered. The impairment of ANP receptor-operated and amiloride-sensitive Na^+ transporter in the BBB may be related to the etiology of hypertension-related encephalopathy.