Atrial Natriuretic Peptide-Induced Inhibition of Amiloride-Sensitive $^{22}\text{Na}^+$ Uptake into Cerebral Capillaries of Spontaneously Hypertensive Rats

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Abstract- $^{22}\text{Na}^+$ uptake into capillaries isolated from the cerebral cortex of adult (20- to 26-week-old) sustained-hypertensive spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) was compared with findings in age-matched Wistar Kyoto rats (WKY). In the presence of 1.0 mM ouabain, 1.0 mM furosemide and 2.0 mM LiCl, $^{22}\text{Na}^+$ uptake into the isolated cerebral capillaries of WKY and SHR was significantly reduced to 38% and 65% of the control values, respectively, when 0.1 μM α -rat atrial natriuretic peptide (rANP) was added to the uptake buffer. The rANP-induced inhibition observed in SHR was significantly less, as compared with that in the WKY. Noteworthy was the observation that the Na^+ uptake into the cerebral capillaries of SHRSP was not inhibited by rANP. As this peptide is thought to regulate amiloride-sensitive Na^+ transport from the blood to brain by interacting with specific receptors, the present finding may relate to the etiology of dysfunction of the blood-brain barrier, in the presence of hypertension.

Water-electrolyte permeability across the blood-brain barrier seems to be regulated by four systems, amiloride-sensitive Na^+ transport and furosemide-sensitive $\text{Na}^+\text{-Cl}^-$ cotransport on the luminal membrane of the capillary endothelium, and Na^+ , K^+ -ATPase and Na^+/H^+ exchange on the antiluminal membranes (1). Amiloride-sensitive Na^+ transport and furosemide-sensitive $\text{Na}^+\text{-Cl}^-$ cotransport take up Na^+ from the circulating blood, and the Na^+/H^+ exchange regulates the influx of Na^+ from the brain into the epithelium. The amiloride-sensitive Na^+ transport in renal cells is inhibited by atrial natriuretic peptides (ANPs) (2,3), presumably by interacting with the ANP- R_1 receptor linked to guanylate-cyclase (4).

We obtained evidence for the existence of specific ^{125}I - α -rat atrial natriuretic peptide (1-28)[ANP-(99-126), ^{125}I -rANP] binding sites, candidates of physiologically active receptors in cerebral capillaries isolated from the rat cerebral cortex (5). The number of specific ^{125}I -rANP binding sites in the cerebral capillaries of 20-week-old spontaneously hypertensive rats (SHR) was also found to be significantly lower than that of age-matched normotensive Wistar Kyoto rats (WKY) (6). Thus, a possible role of ANP receptors

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in the etiology of dysfunction of the blood-brain barrier complicated with hypertension would have to be considered. The amiloride-sensitive Na^+ uptake into cells was selectively measured in vitro, using $^{22}\text{Na}^+$ and uptake medium containing ouabain, an inhibitor of Na^+ , K^+ -ATPase, furosemide, an inhibitor of Na^+ - Cl^- cotransport, and LiCl , an inhibitor of Na^+ / H^+ exchange (7). We investigated the effect of rANP on the in vitro amiloride-sensitive $^{22}\text{Na}^+$ uptake into the cerebral capillaries isolated from adult WKY, SHR and stroke-prone SHR (SHRSP).

Fifteen male adult (20- to 26-week-old) WKY, SHR and SHRSP maintained and bred at the Animal Laboratory Center of Nagasaki University School of Medicine were used. Systolic blood pressure (mmHg) of these WKY, SHR and SHRSP was 134 ± 8 (mean $\pm$ S.E.M., $n=15$), 186 ± 16 ($n=15$) and 235 ± 15 ($n=15$), respectively. Capillaries were isolated from the cerebral cortex using a nylon mesh (153 μm) and a glass beads (60-80 mesh)-column, as described (5,6). Purity of the cerebral capillaries was carefully assessed by phase-contrast microscopy and biochemical criteria, γ -glutamyl transpeptidase to check for the presence of arterioles, venules and glial cells (6). About 200 μg protein of cerebral capillaries per rat could usually be obtained. The capillaries were washed twice with 10 mM Tris-HCl buffer, pH 7.4, containing 250 mM sucrose, 1.4 mM NaCl, 5.0 mM glucose, 40 $\mu\text{g}/\text{ml}$ bacitracin, 4 $\mu\text{g}/\text{ml}$ leupeptin, 2 $\mu\text{g}/\text{ml}$ chymostatin, 100 KIU/ml aprotinin, 1.0 mM ouabain, 1.0 mM furosemide, and 2.0 mM LiCl, which facilitates Na^+ uptake across the amiloride-sensitive Na^+ transport (7). The capillaries were preincubated in the uptake buffer at 37°C for 30 min in the presence or absence of 0.1 μM rANP (Peptide Institute) (2), and then incubated at 37°C for 90 sec with 110 nCi $^{22}\text{NaCl}$ (1091 mCi/mg, New England Nuclear) in 110 μl of uptake buffer. The capillaries taking up $^{22}\text{Na}^+$ were washed three times with 5.0 ml of ice-cold 0.1 M MgCl_2 and passed through Whatman GF/C filters presoaked with 1.0 M NaCl. The washed filters were assayed for radioactivity, using a γ -counter. Results were expressed as means $\pm$ S.E.M. Differences in the data were assessed by one-way analysis of variance (ANOVA), using the F-test.

The isolated cerebral capillaries (Fig.1) preincubated and incubated in the uptake buffer in the absence of rANP took up about 3 nmol/mg protein of $^{22}\text{Na}^+$, during the 90 sec incubation period (Fig. 2). There were no significant differences in amounts taken up in the absence of rANP between WKY, SHR and SHRSP. When 0.1 μM rANP was added to the uptake buffer, $^{22}\text{Na}^+$ uptake into the cerebral capillaries of WKY and SHR was significantly inhibited. The potency of rANP in inhibiting the uptake in SHR was less than that of WKY. Interestingly, rANP did not inhibit the uptake into the cerebral capillaries of SHRSP.

The present evidence of poten-

Fig. 1. Phase-contrast microscopic observation of cerebral capillaries obtained from adult Wistar Kyoto rat. Bar = 50 μm .

Fig. 2. Inhibition of $^{22}\text{Na}^+$ uptake into the cerebral capillaries of adult WKY, SHR and SHRSP across the amiloride-sensitive Na^+ transport by $0.1 \mu\text{M}$ rANP. The columns represent the means $\pm$ S.E.M. of 10 determinations. ** $P < 0.01$, significant differences between the values obtained in the presence (hatched columns) and absence of rANP (open columns). ☆☆ $P < 0.01$, significant differences between the values of adult WKY, SHR and SHRSP obtained in the presence of rANP.

cy of rANP in inhibiting the amiloride-sensitive Na^+ influx into the cerebral capillaries of SHR could relate to decreases in the number of specific ^{125}I -rANP binding sites (6). This seems pertinent to the existence of ANP receptors-operated amiloride-sensitive Na^+ channel in the blood-brain barrier. Although there is little information on the nature of ANP receptors in the cerebral capillaries of SHRSP, of particular interest was our observation that there was no inhibitory effect of rANP on the Na^+ uptake in this animal model of cerebral apoplexy. Dysfunction in water-electrolyte permeability across the blood-brain barrier in sustained-hypertensive SHR is an event which precedes hypertensive encephalopathy and cerebral apoplexy (8). Taken together, the amiloride-sensitive Na^+ transport coupled with ANP receptors may participate in the pathophysiology related to dysfunction of the blood-brain barrier complicated with hypertension.

There is a significant increase in the Na^+ influx across the cell membranes of lymphocytes of SHRSP, probably under the control of a genetically-determined factor (9). Ek and Deth most recently reported the high activity of aortic Na^+/H^+ exchange in adult SHR (10). Thus, our findings of an abnormality in the amiloride-sensitive Na^+ transport coupled with ANP receptors are pertinent to etiological factors of hypertension.

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