25) Effect of Verapamil on Cardiac and Renal α-Adrenoceptors in Spontaneously Hypertensive Rats
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The greater inhibition of pressor responses to noradrenaline by Ca-antagonists has been observed in the blood vessels of spontaneously hypertensive rats (SHR, Okamoto and Aoki) than the normotensive controls. These effects by Ca-antagonists may be due to inhibiting Ca++ flux through potential-dependent and possibly also through receptor-mediated channels in the plasma membrane. Recent studies suggest that the Ca++ antagonist, such as verapamil, might interact with α-adrenoceptors. We have reported the increase in renal α-adrenoceptors in SHR. In the present study, effect of verapamil on renal α₁- and α₂-, and also cardiac α₁-adrenoceptors in SHR were observed using radioligand binding technique.

MATERIAL AND METHODS
In this experiment, 7 weeks-old male SHR and Wistar-Kyoto rats (WKY) were used. The plasma membrane fractions (105,000g fraction) of the heart and the kidney were prepared by ultracentrifugation method. For the α₁- and α₂-adrenoceptors binding assay, membrane fractions (0.2mg protein) were incubated in buffer solution (0.25M Sucrose, 1mM MgCl₂, 5mM Tris-HCl, pH 7.4) with 0.2-5.0nM [3H]-prazosin or [3H]-yohimbine, in the absence or presence of 10μM verapamil, for 15min. at 25°C. To examine the effect of verapamil on specific binding of [3H]-prazosin and [3H]-yohimbine, the membranes were incubated with 0.5nM of radioligands and 1×10⁻⁸M~1×10⁻³M verapamil for 15min. at 25°C. Non-specific binding was determined with 10μM prazosin for [3H]-prazosin and 10μM phentolamine for [3H]-yohimbine. After incubation, the membrane-bound radioligand was collected on a glass fiber filter and counted in liquid scintillation counter. The protein was determined by the method of Lowry et al.

RESULTS
The concentration of renal α₁- and α₂-adrenoceptors in SHR was significantly increased at 7 weeks (68.0±4.6 fmol/mg protein, 59.5±5.6fmol/mg protein, mean±SEM, P<0.05), as compared with age-matched WKY (53.8±3.2, 50.9±5.4). No significant difference was found in the cardiac α₁-adrenoceptor concentration between SHR and WKY. The Kd values of cardiac and renal α-adrenoceptors in SHR were not different from the WKY. Verapamil (10μM) inhibited [3H]-prazosin or [3H]-yohimbine binding to cardiac α₁-, renal α₁-, and α₂-adrenoceptors in both SHR and WKY. The increase in the Kd values (Cardiac α₁-, renal α₁-, renal α₂-adrenoceptors) by verapamil was greater in SHR (0.48±0.021nM, 1.48±0.12nM, 5.3±0.41nM) than in WKY (0.38±0.041, 1.25±0.18, 4.2±0.24, respectively, P<0.05). KI values of verapamil to these α-receptors of SHR were significantly lower than those of WKY (SHR: 2.1±0.25μM, 1.2±0.08μM, 1.3±0.09μM vs WKY: 3.8±0.32, 2.2±0.09, 2.6±0.15, respectively, P<0.05).

DISCUSSION
The renal α₁- and α₂-adrenoceptor concentration of 7 weeks old SHR was increased, as shown in our work. Verapamil inhibited [3H]-prazosin and [3H]-yohimbine binding to cardiac α₁-, renal α₁- and renal α₂-adrenoceptors in both SHR and WKY. In the presence of 10μM verapamil the Kd values of α-receptors were markedly increased in SHR, as compared with WKY. These results suggest that α-adrenoceptors in SHR are more sensitive to verapamil, and it might be due to the higher sensitivity to the inhibitory effect of Ca++ flux through the receptor-mediated Ca++ channels, by verapamil in SHR.