55) Reduced Number of β -Adrenergic Receptors in the Myocardium of M-SHRSP. Hiroshi Ikawa, Yukiko Miki and Aritomo Suzuki. Department of Pharmacology, Kinki University School of Medicine, Osaka-fu 589.

INTRODUCTION: It has been reported that Malignant-SHRSP (M-SHRSP, Okamoto et al, 1984) developed from SHRSP dies by stroke at the early stages. In view of the severity of the hypertension in M-SHRSP, early and marked myocardial changes would be anticipated. The present study was undertaken to examine myocardial β -adrenergic receptors in M-SHRSP using the radioligand [^3H]-dihydroalprenolol (DHA).

MATERIALS AND METHODS: M-SHRSP, SHRSP and WKY at 10 weeks of age were used. All subsequent procedures were carried out at 0-4°C. Rat ventricles were homogenized with 10 volumes of cold homogenate buffer (0.25M sucrose, 1mM MgCl_2 , 5mM Tris-HCl, pH 7.4) by a Polytron PT-10 at a setting of 8 for 30 sec. The homogenate was centrifuged at 480g for 10 min. The pellet was discarded and the supernatant was centrifuged at 30000g for 10 min. The pellet was washed twice in cold incubation buffer (10mM MgCl_2 , 50mM Tris-HCl, pH 7.4) and was finally resuspended at a concentration of 3-5mg of protein/ml in incubation buffer. For (-)-[^3H]dihydroalprenolol ([^3H]DHA: 70Ci/mmol, Amersham) binding, membrane suspension (300-500 μg protein) was incubated with varied concentration of [^3H]DHA (1nM-10nM) in the presence and absence of 10 μM ($\pm$)-propranolol for 15 min at 37°C. Incubation was terminated by diluting the samples with 1ml of cold incubation buffer, immediately followed by vacuum filtration through Whatman GF/C glass fiber filters. The filters were rapidly washed twice with 6ml of the same buffer and then dried and added to scintillation fluid (New England Nuclear, Econofluor). Filters were counted in a Packard liquid scintillation spectrometer at an efficiency of 40 %. Specific binding was defined as the difference in binding in the presence and absence of 10 μM ($\pm$)-propranolol. Specific binding for [^3H]DHA was 50 to 75 % of total binding. Binding data were assessed by the method of Scatchard.

RESULTS AND DISCUSSIONS: The blood pressures of M-SHRSP, SHRSP and WKY at 10 weeks were 240, 200 and 130mmHg, respectively. The heart weight to body weight ratios of M-SHRSP and SHRSP were higher than that of WKY. The yields of final membrane preparation per Gm of initial heart weight from M-SHRSP, SHRSP and WKY were similar at about 5mg of membrane protein/Gm of initial wet weight. Myocardial β -receptor numbers from M-SHRSP were significantly decreased compared with those from WKY and SHRSP. And myocardial β -receptor numbers from SHRSP were significantly decreased compared with those from WKY. They were 36.7 ± 1.5 (mean $\pm$ S.E.) fmol/mg protein from M-SHRSP and 41.7 ± 1.0 from SHRSP, and 50.3 ± 1.2 from WKY, respectively. The dissociation constant (K_d) was not significantly different among the three groups. They were 5.26 ± 0.24 nM from M-SHRSP and 5.30 ± 0.24 from SHRSP, and 5.44 ± 0.21 from WKY, respectively. Recently, it has been reported that the adrenergic agonist induced the decrease of β -receptor numbers (down regulation). As a result of this study, we supposed that increased sympathetic drive may exist in M-SHRSP and SHRSP.