Morphological and biochemical studies on hypertrophied SHR myocardium mitochondria and plasma membrane. Mikinori Torii, Hiroyuki Ito and Tsuneyuki Suzuki. Department of Pathology, Kinki University School of Medicine, Osaka 589.

In a basic experiment designed to elucidate the pathogenesis of SHR myocardial disorders, we have already examined age-related SHR myocardium enzyme activity changes and revealed that hypertrophied SHR myocardium suffered from mitochondria and plasma membrane abnormalities. (Torii et al., Jpn Heart J 29; 534, 1988) In order to confirm these myocardial abnormalities, in this study we examined mitochondria morphological changes as well as lipid peroxidation resulting from adriamycin administration.

Male SHR and WKY hearts were excised and examined at 6 or 16 weeks of age. 1) Mitochondrial morphometrical and biochemical study: After exception of the epicardium, the left ventricle and septum were isolated, minced and homogenized in 10-fold 0.25 M sucrose-10mM Tris-HCl buffer (pH 7.4, 4°C). The mitochondrial fraction was obtained by, following centrifugation at 1,000 xg for 10 min., further centrifugation at 10,000 xg for 10 min. One portion of the fraction was fixed in 3% glutaraldehyde before being postfixed in 2% OsO4. Morphometrical analysis on cross section area of mitochondria was carried out for electron microscopic pictures using image analysis system (Kontron). The other portion was resuspended in the same buffer and used in an assay for isocitrate dehydrogenase (ICDH), a mitochondria-related enzyme. 2) Lipid peroxidation study: Sixteen-week old SHR and WKY were given adriamycin (10 mg/kg, i.v.) then sacrificed on the 4th or 7th day. Control rats were given physiologic saline. The hearts were prepared as above and homogenized in 0.1 M KCl-50 mM Tris-HCl buffer (pH 7.5). Hunter's thiobarbituric acid (TBA) method was carried out on microsomal fractions by centrifugation at 105,000 xg for 35 min. At the same time, Na/K-ATPase and 5'-nucleotidase activities, which are plasma membrane-related enzymes, were examined according to Lamer's method and a modified Song and Bodansky's method, respectively.

At 6 weeks of age, ICDH activity was significantly higher in SHR than in WKY. In both strains at 16 weeks of age, ICDH activity had decreased significantly compared to those in 6 weeks of age and SHR activity was found to be lower than that of WKY. The results of the morphometrical analysis at 6 weeks of age showed that mitochondria size were widely distributed in both strains, but these histogram patterns were narrower at 16 weeks of age. At 6 weeks of age, the average size of SHR mitochondria was significantly larger than that of WKY but at 16 weeks of age this significant difference had disappeared. These results coincided well with those on ICDH activity. On the other hand, compared to their respective controls, Na/K-ATPase activities were significantly lower in both the SHR and the WKY adriamycin-treated groups as of the 4th day of administration. Treated SHR group 5'-nucleotidase activity was also significantly lower than it was in the control, but no differences were found for the WKY experimental and control groups. Also as of the 4th day, no increase in microsomal fraction TBA-reacting substances were found in either the treated SHR or treated WKY group. However, TBA-reacting substances had increased in both the treated SHR and the treated WKY group as of the 7th day, the results being significantly higher for treated and control SHR but not so for WKY. These results suggest the enhancement of lipid peroxidation in myocardium plasma membrane of aged SHR, since TBA-reacting substance is generally accepted as a marker of lipid peroxidation. These results indicate that the alteration not only in mitochondria but also in plasma membrane of myocardial cells, especially lipid peroxidation of plasma membrane, could be one of causative factors for myocardial damage in SHR.