10) Elastase in Aorta in SHR-SP. Eiji Yamada, Hideo Kataoka, Fumitada Hazama, Shigeru Amano, Masakiyo Sasahara and Kalula Kayembe. Department of Pathology, Shiga University of Medical Science, School of Medicine, Otsu, 520-21.

Decrease of alkaline insoluble elastin in aorta in SHR has been reported. In attempts to obtain information concerning the elastase in the development of hypertensive vascular changes, we investigate the aortic elastase with biochemical and immunohistochemical studies.

MATERIALS AND METHODS For biochemical study we used male SHR-SP aged 20 weeks and age-matched WKY. Immediate after sacrifice, the aortas were removed from the heart to coeliac artery. The adherent fibrous and fatty tissues were removed, and the intima and media fragments were prepared. After washing in ice-cold saline 3 times, the fragments were cut into small pieces, weighed and homogenized in distilled water using a Teflon homogenizer. The 5% homogenates were centrifuged at 1800 rpm for 20 min. The resulting supernatants were used as the enzyme samples. The temperature was maintained at 4°C throughout these procedures. Elastase activity was assayed by Bieth's method using Suc-(Ala)₃-pNA as a substrate. Elastase activity was expressed as E410nm/hr/10 mg tissue weight. For immunohistochemical study, SHR-SP and WKY aged 24-32 weeks were used. They were perfused with saline from the left ventricle, followed by buffered formalin (pH 7.4). Thoracic aortas were removed and embedded in paraffin. Elastase localization was examined with PAP method using rabbit anti-rat-pancreatic elastase (Eisai Co. Ltd.).

RESULTS AND DISCUSSIONS Elastase activity in SHR was 0.172 ± 0.009 /hr/10 mg tissue weight and that in WKY 0.128 ± 0.031 /hr/10 mg tissue weight. Aortic weight of SHR was 44.5 ± 6.5 mg and that of WKY 30.3 ± 3.7 mg. These differences were statistically significant between SHR and WKY. Total aortic elastase in SHR was 2-fold higher than that in WKY. These results correspond well with acid phosphatase activity, which is one of lysosomal marker enzymes, in our previous report (Yamada et al. Jpn. Heart J. 21, 1980). It is said that elastase more or less specifically hydrolyzes interstitial elastin. Decrease of alkaline insoluble elastin in SHR has been reported by Ito et al. (Jpn. Heart J. 20, 1979). In immunohistochemical study elastase was demonstrated in endothelial and medial smooth muscle cells as granular brown pigments and some times along membranes of intracytoplasmic vacuoles. These observation indicate elastase were localized in lysosomes of vascular cells. Subendothelial spaces adjacent to elastica interna also showed elastase localization. Intimal thickening, due to proliferation of modified smooth muscle cells, is one of characteristic findings in the arteriosclerotic arteries. Therefore, elastase plays some important roles in migration of medial smooth muscle cells from media to intima by digesting the internal elastic lamina. Increased elastase activity in SHR seems to indicate also an accelerated metabolism in arterial elastin. Pancreatic elastase pass into vascular tissue via the circulation is known, and it is still somewhat controversial whether the demonstrated aortic elastase is vascular origin or not. However, Nakao et al detected elastinolytic activity in rat aortic smooth muscle cells in culture (Atherosclerosis, 36, 1980). This report suggest the existence of aortic elastase in SHR.