1) Changes by hypertension and hypercholesterolemia in arterial enzyme activities in the rat. Takako Tomita, Yasufumi Shirasaki, Yoshiharu Takiguchi, Keizo Umegaki, Norihisa Taniguchi and Eiichi Hayashi. Department of Pharmacology, Shizuoka College of Pharmaceutical Sciences, Shizuoka, 422

Hypertension as well as hyperlipidemia is one of the major risk factors in the genesis of atherosclerosis. The enhancing effect of hypertension on the development of atherosclerosis in man and experimental animals has been widely documented. However, little is known about the mechanism by which hypertension aggravates atherosclerosis. It has been observed that hypertension accompanies changes in lysosomal hydrolase activity in the aorta. These arterial lysosomal changes were also found to occur in atheromatous vessels in a number of experimental animals and in man. Consequently, we assumed that a link between hypertension and atherogenesis might underlie these arterial enzymes. As atherosclerosis is a disorder of cholesterol ester(CE) metabolism in the aorta, an imbalance between hydrolysis of CE by cholesteryl esterase(CEase) and reesterification of cholesterol by ACAT induces undesired results on the arterial wall. It has been postulated that the hydrolytic action of LPL on triglyceride-rich lipoproteins (chylomicrons and VLDL) with a concomitant generation of high concentrations of CE-enriched remnants at the arterial endothelial surface, also plays a role in the initiation of atherosclerosis. The study presented here, was undertaken to clarify the mechanism by which hypertension accelerates atherosclerosis, with regard to arterial lipid metabolism. For this purpose, spontaneously hypertensive rats were treated with hypotensive drugs and changes due to hypertension in acid CEase and other lysosomal enzyme activities were examined. Subsequently, the effects of other experimental hypertension on these enzyme activities were compared with those of spontaneous hypertension. Lastly, the effects of hypertension superimposed by hypercholesterolemia were examined.

Effects of spontaneous hypertension: In order to investigate effects of hypertension, one group of spontaneously hypertensive rats were started on a treatment of hypotensive drugs at weaning to inhibit a development of high blood pressure. This treatment was continued for 4 months, while another group had no such treatment. The blood pressure in SHRSP was significantly lowered to between 120-140 mmHg during the experimental period by the hypotensive treatment, whereas the blood pressure of nontreated SHRSP was, on average, 229 mmHg at the end of the experiment. CEase activity in the aorta from the treated group was significantly higher than that from the nontreated group. The enzyme activity in the inferior vena cava which is not hemodynamically affected, however, was unchanged due to the treatment. In a striking contrast, the activity of N-acetyl- β -D-glucosaminidase(NAGA) in the aorta was markedly increased in the hypertensive group, but there was no difference in the activity of the vein. Acid phosphatase(AP) activity did not appear to fluctuate following the treatment, either in the aorta or the vein. This observation in SHRSP was confirmed in SHR. The effects of a long term administration of hypotensive drugs on these enzyme activities were not observed in normotensive WKY in accordance with a only slight change in the blood pressure. These results suggest that a long term administration of the hypotensive drugs per se does not affect arterial enzyme activities, and that changes caused by the treatment were due to the hypotensive effect of these drugs. A consequent change in aortic cholesterol was observed following a long duration of hypertension. There was a 108% increase in CE and 43% increase in free cholesterol in the aorta of nontreated SHRSP, compared with that of the treated group after a 5 month experimental period, while a 95% and 14% increase in esterified and free cholesterol was observed in the non-treated SHR, compared with those of the treated SHR after a 6 month experimental period.

Effects of experimental hypertension: The response of aortic enzyme activities to spontaneous hypertension was then compared with that of renal hypertension and DOCA-salt hypertension. The blood pressure of the DOCA-salt hypertensive group was increased to an average of 240 mmHg by the end of the one month experimental period. In contrast to the results obtained with spontaneously hypertensive rats, CEase activity, both in the aorta and the inferior vena cava from the DOCA-salt hypertensive group, was significantly elevated by 49% and 26%

respectively, compared to those in the control group. AP activity was also significantly increased due to the hypertension both in the aorta and the vein. These results suggest that humoral factors were the cause of the enzyme fluctuation in DOCA-salt hypertension, while hemodynamic effects were evident in spontaneous hypertension. In order to see the sole effect of NaCl given together with DOCA to induce hypertension, 1% NaCl was given to rats instead of water for 2 months. Despite there being no difference in blood pressure between the NaCl group and the control group, aortic and venous CEase activity was enhanced by 25% and 30% respectively due to NaCl loading. NAGA and AP activities were also increased both in the aorta and the vein of the NaCl group. These results strongly indicate that humoral factors including NaCl were involved in the increase of lysosomal enzyme activities. The changes by renal hypertension (one clip-one kidney & one clip-two kidney) appeared to be due both to hemodynamic and to humoral effects. There were distinct differences in the responses of lysosomal enzymes to hypertension with the three different etiologies.

Effects of hypertension superimposed by hypercholesterolemia: Although hypertension aggravates atherosclerosis, hypertension per se, is unlikely to induce atherosclerosis in the absence of other atherogenic factors. Studies so far reported in men and experimental animals, proved that hypertension accelerates atherosclerosis when hypertension accompanies hyperlipidemia. In this view it is interesting to examine the effects of hypertension superimposed by hyperlipidemia on arterial enzyme activities. Firstly, the sole effect of hypercholesterolemia under a normotensive pressure were investigated by giving a diet with and without a supplement of cholesterol to WKY for a 4 month's experimental period. The serum cholesterol level reached a maximum (1,700 mg%) at 10 weeks. Aortic CEase, NAGA and AP activities were not changed by hyperlipidemia alone, while the LPL activity was significantly increased. The effects of hypertension superimposed by hypercholesterolemia vs. hypertension alone were examined in female SHRSP which had been similarly treated. The blood pressure of both groups was about 220 mmHg at the conclusion of the experiment. The serum cholesterol level in the cholesterol fed group rose over 2,000 mg% at 10 weeks and remained at this level. CEase activity increased slightly but significantly in cholesterol fed SHRSP, and LPL activity increased also. Accompanying the change in the enzyme activities, the aortic CE content was 2.5 fold higher in the cholesterol fed group. The effects of hypercholesterolemia superimposed by hypertension vs. hypercholesterolemia alone were then examined in male and female SHRSP. Both groups were given the cholesterol diet with and without hypotensive drugs for 4 months. The blood pressure was, on average 205 mmHg in the hypertensive group, and on average 132 mmHg in the hypotensively treated group. Serum cholesterol was about 1,600 mg% in both groups at the end of the experiment. The LPL and NAGA activities are markedly increased in the hypertensive group, whereas CEase and AP activities were unchanged.

In summary, aortic CEase activity decreases through hypertension, remains unchanged through hypercholesterolemia alone, increases through hypertension superimposed by hypercholesterolemia. On the other hand, LPL activity is always greatly enhanced by hyperlipidemia, both in a normotensive state and in a hypertensive state, but not by hypertension alone. The increase in LPL activity was also observed in the inferior vena cava. By contrast, an increase in NAGA activity can only be observed following hypertension, regardless of the presence or absence of hypercholesterolemia. This change does not occur in the vein. AP shows no response to any stimulation applied in this study within the experimental period. In hypertension superimposed by hypercholesterolemia, the influx of lipoprotein is facilitated as a consequence of an elevation in serum lipids and in LPL activity. A small decrease in aortic CEase following hypertension may not impair the lipoprotein metabolism under a normolipemic condition. When an enhancement of aortic CEase activity following an increased influx of lipoprotein, is inhibited by hypertension, however, a relative deficiency of this enzyme activity to the uptaken lipoproteins may occur and lead to an accumulation of CE in the aorta after a long duration of these conditions.