49) Lipo- and Apo-proteins in SHRSP---Isolation, Characterization and Quantitation of Apo A-I and Apo E in SHRSP. Hiroshi Ogawa, Chiyo Shiota, Tomoyo Nishikawa, Shinichi Fukushima and Sukenari Sasagawa. Department of Hygiene, Kinki University School of Medicine, Osaka-fu 589.

It is generally accepted that high density lipoprotein(HDL) is an anti-atherogenic factor and that there is a negative correlation between the concentration of HDL-cholesterol and the incidence of coronary heart disease. Both apo A-I and apo E are major components of HDL in rats and have important functions in lipoprotein metabolism. Apo A-I plays an important role as cofactor for lecithin: cholesterol acyltransferase(LCAT) and apo E as recognition site for receptor-mediated hepatic removal of lipoproteins and for pick-up of cholesterol from peripheral tissues. In the present experiment, 1) we isolated and characterized apo A-I and apo E from SHRSP which is known as a good model of studies on atherosclerosis, 2) we determined serum concentrations of apo A-I and apo E in SHRSP and WKY by using electroimmunoassay method.

MATERIALS AND METHODS: Male SHRSP and WKY were used to collect blood for isolation of apo A-I and apo E. Male 3 week-old(n=8) and 9 week-old(n=8) SHRSP and male age-matched WKY(n=8 each) were used to quantify serum concentrations of apo A-I and apo E. Blood was obtained from jugular vein under ether anesthesia after fasting overnight, but without fasting in the case of 3 week-old rats. Lipoprotein mixtures(VLDL+LDL+HDL) were floated by ultracentrifugation at $d < 1.21$ g/ml, followed by delipidation with ethanol-ether(3:1,v/v) mixture. Apoprotein mixtures obtained were fractionated into unretained fraction(apo A-I frac.) and retained fraction(apo E frac.) with Heparin-Sepharose affinity chromatography. For isolation of apo A-I, apo A-I frac. was purified by Sephacryl S-200 and finally FPLC system(Mono Q, Pharmacia). For isolation of apo E, apo E frac. was purified by DEAE-Sephadex A-25, followed by FPLC system(Mono Q, Pharmacia). SDS-gradient PAGE was carried out as mentioned at 19th this council. Isoelectric focusing(IEF) was performed on polyacrylamide slab-gel(T=5% and C=3%) containing 7.1M urea and 6%-Pharmalyte(pH 4-6.5, Pharmacia). The stained gels were scanned by laser densitometer(LKB 2202 ULTROSAN). Serum concentrations of apo A-I and apo E were determined by electroimmunoassay(EIA) method as described in the accompanying paper.

RESULTS AND DISCUSSION: At the final purification with FPLC system, apo A-I gave two peaks(apo A-I-1 and apo A-I-2) which had the same amino acid composition and showed the same R_f value on SDS-gradient PAGE, but showed different pattern of isoforms in IEF. No distinct differences were observed between SHRSP and WKY when apo A-I isolated from each strain was characterized separately. Apo E was eluted as single peak in FPLC system, and apo E from SHRSP was focused in the same regions as apo E from WKY except extra bands with higher pI values, which may be due to a slight contamination in apo E from SHRSP. It is well known that apo E from patients with broad- β -disease(Type III hyperlipoproteinemia) is usually associated with the special apo E phenotype(E2/2), and is characterized by the deficiency in E-3 and E-4 bands. In SHRSP, there was no deficiency of apo E isoforms compared with those of WKY in IEF. So, there might be no lipid disorder based on deficiency of apo E isoforms in SHRSP. Moreover, we prepared antibody against apo A-I-1 and apo E respectively. Apo A-I-2 reacted with antiserum to apo A-I-1, which indicated that apo A-I-2 had the same antigenicity as apo A-I-1.

There was no significant difference in the concentration of serum apo A-I between SHRSP and WKY at the age of 3 weeks. But, at the age of 9 weeks, significant difference was observed (SHRSP; 36.3 ± 1.6 mg/dl against WKY; 53.2 ± 1.7 mg/dl). At this age, hypertension in SHRSP was not established but progressed. So, the concentration of apo A-I in SHRSP might be related to the increase of blood pressure. On the other hand, serum concentrations of apo E in 3 and 9 week-old SHRSP were much lower than those in age-matched WKY. This decreased contents of apo E in SHRSP in comparison with WKY suggest that there might be genetically impaired biosynthesis of apo E and/or decreased secretion of HDL containing apo E as its major component in SHRSP.