Reduced spontaneous relaxation following maximum contraction by Angiotensin II in endothelium-denuded aorta from SHRSP. Satoshi Mashiko, Takeshi Onda, Megumi Hamano, Isao Tomita, *Masahiko Ikeda and *Takako Tomita. University of Shizuoka School of Pharmaceutical Sciences, *Graduate School of Health Sciences, 52-1 Yada, Shizuoka, 422, Japan.

Angiotensin II (AngII) induces vascular smooth muscle contraction through specifically activating phospholipase C-mediated breakdown of phosphatidylinositol diphosphate (Alexander et al. *Hypertens* 7, 447, 1985). The products, inositol triphosphate and diacylglycerol, then increases intracellular Ca^{2+} by releasing Ca^{2+} from the endoplasmic reticulum, and activates protein kinase C (PK-C), respectively. It is recently reported that PK-C stimulates Ca^{2+} -ATPase activity in the sarcolemmal reticulum through its direct phosphorylation and Ca^{2+} -uptake into the vesicles (Fukuda et al. *J. Biochem.* 108, 629, 1990). We found that platelet dysfunction in SHRSP result from defective protein (P47) phosphorylation by PK-C (Tomita et al. *Hypertens.* 14, 304, 1989), and repeated stimulation of mesenteric vascular beds from SHRSP shows less contractile responses than that from WKY, suggesting the impaired Ca^{2+} -pump in SHRSP vascular smooth muscle. In this study, contractile response to AngII was examined in connection with changes of intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) in endothelium-denuded aortic rings from SHRSP and WKY attempting to investigate roles of PK-C in regulation of vascular tone.

SHRSP and WKY were maintained under a specific pathogen free condition by brother-sister breeding in our laboratory. Ring segments (3mm) of thoracic aorta were prepared from male SHRSP and WKY at 12-14 weeks of age and the endothelium was removed by gently rubbing with a stainless steel wire. Aortic rings were mounted in organ baths that contained Tyrode's solution (pH 7.3, 37°C) aerated with O_2/CO_2 gass (95:5), and tension was measured isometrically at 2g of resting tone. Ring segments were loaded with $10\mu\text{M}$ fura-2/AM in the presence of 0.08% cremophor EL (Nacalai tesque, Kyoto) for 6-8hrs at room temperature, and then ring segments were rinsed with Tyrode's solution. They were mounted in a muscle bath attached to a fluorometer (CAF-100, Japan Spectroscopic, Tokyo, Japan). Tension and $[\text{Ca}^{2+}]_i$ were then simultaneously monitored. The magnitude of spontaneous relaxation at 5min following the maximum contraction was expressed as 100% of AngII (10^{-6}M)-induced maximum contraction. Time-dependent change in $[\text{Ca}^{2+}]_i$ was expressed as 100% of AngII (10^{-6}M)-induced maximum $[\text{Ca}^{2+}]_i$.

AngII (10^{-9}M - 10^{-6}M) induced dose-dependent transient contraction in denuded aortic ring preparation both from SHRSP and WKY. The maximum contraction in SHRSP was in a similar degree to that in age-matched WKY at any tested concentrations. However, the subsequent spontaneous relaxation was impaired in SHRSP aortic rings compared with that of WKY; the relaxation at 5min after the maximum contraction was 24% attenuated. An addition of 1-oleoyl-2-acetyl-sn-glycerol (OAG; $100\mu\text{M}$), a specific PK-C activator, to organ baths when SHRSP aortic rings were maximally contracted by AngII (10^{-6}M), relaxed it as much as WKY rings, while the relaxation in WKY rings was not altered by an addition of OAG. Fura-2/AM loading for 4-6hrs in thoracic aorta did not change contractile response in both preparations. AngII (10^{-6}M) induced a sharp peak of $[\text{Ca}^{2+}]_i$ with subsequent development of vascular tension. $[\text{Ca}^{2+}]_i$ reached a peak at approx. 10sec and then decreased; in WKY aortas, $[\text{Ca}^{2+}]_i$ gradually decreased and returned to the basal level at approx. 10min, while in SHRSP aorta, the initial phase of decrease in $[\text{Ca}^{2+}]_i$ was faster than in WKY but the second phase was slow and did not return to the basal level. $[\text{Ca}^{2+}]_i$ 10min after AngII (10^{-6}M)-stimulation was 25.7% higher in SHRSP than that in WKY. There was a correlation between sustained contraction (percentage of maximum contraction) and $[\text{Ca}^{2+}]_i$ (percentage of maximum) 10min after AngII-stimulation ($r=0.926$).

In summary, spontaneous relaxation following AngII-induced maximum contraction was significantly diminished in SHRSP vascular smooth muscle, and OAG restored the impairment. This sustained contraction seemed to be associated with the sustained higher level of $[\text{Ca}^{2+}]_i$. These results, together with our previous findings described briefly above, suggest that sustained contraction in SHRSP vascular smooth muscle might result from defective Ca^{2+} extrusion to the sarcoplasmic reticulum that is regulated by PK-C.