Sustained Contraction to Angiotensin II and Impaired Calcium-Pump Activity in Smooth Muscle from SHRSP. Masahiko Ikeda, Hideya Mizuno, Norihiro Mochizuki, Masaki Tabuchi and Takako Tomita. University of Shizuoka Graduate School of Health Sciences, 52-1 Yada, Shizuoka, 422-8526.

Angiotensin II (AngII) is a potent physiological vasoconstrictor peptide and a growth factor of smooth muscle cells. AngII also induces NO release from the endothelium and relaxes smooth muscle. We have reported that AngII-induced endothelium-dependent relaxation in the thoracic aorta from SHRSP at developmental ages of hypertension was significantly enhanced in comparison with that of age-matched WKY (Mashiko et al., Jpn. Heart J. 1995, 36, 528). Binding of AngII to AT₁ receptor in the smooth muscle cell surface activates PLC- γ which generates the second messengers, inositol-1,4,5-trisphosphate (IP₃) and diacylglycerols (DAG). IP₃ mobilizes Ca²⁺ from endoplasmic reticulum (ER), intracellular Ca²⁺ store and induces a rapid increase in intracellular free calcium ion concentration ([Ca²⁺]_i). This rise in [Ca²⁺]_i mediates the phasic contraction in response to AngII. [Ca²⁺]_i returns to the basal levels by Ca²⁺-sequestration via Ca²⁺-pump in ER, Ca²⁺-efflux via Ca²⁺-pump and Ca²⁺-efflux via Na⁺/Ca²⁺ exchanger in plasma membrane, inducing muscle relaxation. In this study, effects of AngII on contractile responses of smooth muscle, and changes in intracellular free calcium concentration ([Ca²⁺]_i) in cultured vascular smooth muscle cells (VSMC) were compared between SHRSP and WKY.

Male SHRSP at established ages of hypertension (12-17 weeks old) and age-matched WKY were used in this study. The thoracic aorta was excised and aortic rings (3 mm long) were prepared. For endothelium-denuded preparations, the inner surface of the aortic rings were gently rubbed with a steel wire and the preparations were mounted in organ baths filled with Tyrode's solution and tension was measured isometrically at 2 g of resting tone. VSMC were isolated from the thoracic aorta with collagenase, and grown in 35 mm dish with Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS) until reaching confluence. VSMC were harvested with trypsin and grown on glass coverslips. The growth was arrested at confluence by replacing the medium with DMEM without FCS and incubated for 48 hrs. [Ca²⁺]_i was measured with fura-2-loaded VSMC using ARGUS-50 (Hamamatsu Photonics).

Body weight of male SHRSP and WKY used in this experiment at the time of 10 weeks of age was 217±2.6 g (50) in SHRSP and 256±2.4 g (50) in WKY [mean±S.D. (n)]. At this age, their systolic blood pressure was 203±2.0 mmHg (50) in SHRSP and 141±1.0 mmHg (50) in WKY [mean±S.D.

(n)]. Body weight and Blood pressure in SHRSP were significantly different from those in WKY.

AngII (10^{-6} M) induced maximum contraction at approx. 5 min, followed by spontaneous relaxation. The maximum contraction was in a similar degree in SHRSP and WKY, whereas following spontaneous relaxation was significantly attenuated in SHRSP compared with WKY. Relaxation (% as maximum contraction) at 5 min after the peak was $23.3 \pm 3.8\%$ (15) in SHRSP vs $48.0 \pm 3.5\%$ (15) in WKY [mean $\pm$ SEM (n), significance $p < 0.01$]. As the depressor response following the phasic pressor response is assumed to reflect Ca^{2+} -elimination, the above result was suggestive of delayed Ca^{2+} -removal mechanisms in SHRSP. In the second experiment, rings were repeatedly stimulated with AngII (10^{-6} M) at 20 min- and 60 min-intervals. When the first application of AngII induced maximum contraction, rings were washed out and left for 20 min in fresh Tyrode's solution, and the second application was made. The third application was made after a 60 min-rest. There was no significant difference in maximum contractile responses to the first, second and third stimulation in WKY. In contrast, the response to the second stimulation was significantly diminished in SHRSP compared with the first response, and it was restored after a 60 min-rest. These results suggested that Ca^{2+} -sequestration is impaired in SHRSP. Therefore, effects of a Ca^{2+} -pump inhibitor of ER, cyclopiazonic acid (CPA) on contraction were examined. The presence of CPA (10^{-5} M) in Tyrode's solution did not affect the maximum contraction by AngII in both strains. However, spontaneous relaxation following the contraction was diminished to less than the half in WKY by CPA. Contrastingly, CPA did not significantly affect the relaxation in SHRSP. Thus, the relaxation was to a similar degree in SHRSP and WKY in the presence of CPA. These results suggested that ER Ca^{2+} -pump activity in SHRSP was impaired.

Finally, AngII-induced change of $[\text{Ca}^{2+}]_i$ in VSMC from SHRSP and WKY was measured. Basal $[\text{Ca}^{2+}]_i$ in VSMC from SHRSP was significantly increased than that from WKY [86 ± 6 nM (10) in SHRSP, 65 ± 6 nM (10) in WKY, mean $\pm$ SEM (n)]. AngII (10^{-6} M)-induced $[\text{Ca}^{2+}]_i$ increase in VSMC peaked immediately, and then gradually declined. The maximum $[\text{Ca}^{2+}]_i$ was not different between the two strains whereas the rate of decline in $[\text{Ca}^{2+}]_i$ was significantly attenuated in SHRSP compared with WKY. These results supported that Ca^{2+} removal mechanism in VSMC from SHRSP was impaired.

In conclusion, an AngII-induced transient $[\text{Ca}^{2+}]_i$ increase due to IP_3 release from ER and tonic contraction were not altered in SHRSP, whereas the following Ca^{2+} sequestration appears to be impaired in SHRSP, leading to sustained contraction.