29) Contraction and Ca^{2+} -uptake of aortic smooth muscle from Spontaneously Hypertensive Rats. Akihiro Mochizuki, Yoshimichi Yamamoto, Schuta Kondo, Kyuzo Aoki, Takako Mizuno*, and Ken Hotta*. 2nd Dept. of Int. Med, and *1st Dept. of Physiol, Nagoya City Univ. Med. School, Nagoya, Japan 467.

Summary: To find out the cellular role to develop hypertension, the characteristics of membrane systems in vascular smooth muscle to regulate the intracellular Ca^{2+} level were investigated. The differences between Spontaneously Hypertensive Rats (SHR, Okamoto-Aoki) and Kyoto Wistar normotensive rats (KNR) in contractile response and Ca^{2+} influx in the presence and absence of Ca^{2+} -antagonist (verapamil) were analyzed. (i) Aortic strip could induce rapid tension by serotonin (5HT) and noradrenaline (NA) in Ca^{2+} -free medium but not by K^+ . (ii) SHR aortic strips developed larger tension than KNR in normal physiological salt solution (PSS). (iii) SHR aortic strips developed tension was more inhibited by verapamil than KNR. (iv) Ca^{2+} influx in aortic tissue from SHR was smaller than that from KNR, and verapamil inhibited Ca^{2+} uptake in SHR more greatly than in KNR. These findings suggest that the membrane systems of SHR aortic muscle have an abnormal function.

Introduction: Many evidences have been reported on the abnormal function of vascular smooth muscle which contributes to the development of hypertension. The tension developed is probably a reflection of the myoplasmic free Ca^{2+} level in the muscle cell. We tried to characterize the membranes of vascular smooth muscle as a regulatory site for myoplasmic Ca^{2+} level and to clarify the differences of its functions between normotensive and hypertensive rats.

Methods: Age matched male SHR and KNR from 10 to 14 weeks of ages were used. The isolated aortae were cut helically. The strip was incubated in Bohr's physiological salt solution (PSS) bubbled with 95% O_2 and 5% CO_2 under a tension of 4g at 37°C for 2 hours. Isometric tension developed in high K^+ medium, 5HT and NA were measured utilizing a force transducer, amplifier and conventional recorder. For Ca^{2+} influx experiments, small pieces of aortic tissues were exposed to a PSS containing ^{45}Ca for 15 min. The sample was treated with 10 mM LaCl_3 in HEPES buffer and washed, solubilized and assayed for radioactivity. The sample was treated with Ca^{2+} -free PSS containing 2.5×10^{-6} M 5HT twice followed by washing with Ca^{2+} -free PSS, prior to the measurements. This procedure removes most of the intracellularly stored Ca^{2+} .

Results: K^+ induced tension appeared in the presence of Ca^{2+} in the medium and not in the absence of Ca^{2+} . 5HT and NA induced tension, however, induced approximately 2/3 to 1/2 of the tension developed in PSS. NA induced initial fast phasic and slow tonic tension in the aortic strip in PSS. Slow tonic component disappeared in Ca^{2+} -free PSS. Aorta from SHR developed larger tension than that from KNR in PSS. When K^+ , 5HT and NA was added in low and normal Ca^{2+} medium, a curve of tension development has no difference between SHR and KNR. The tension development by various stimulation was inhibited by verapamil. Verapamil suppress fast and slow component and has a effect of both Ca^{2+} influx and release. Verapamil inhibited contraction of the aortic strip from SHR more strongly than that from KNR in the presence of Ca^{2+} and the vasostimulators. After incubation in Ca^{2+} -free PSS tension development induced by the stimulant is inhibited more greatly in SHR aortic strip than KNR. Ca^{2+} influx taken from Ca^{2+} in the medium was smaller in SHR aortic muscle cell than KNR. The inhibitory effect of verapamil on Ca^{2+} influx was stronger in SHR than KNR.

Discussion: Aortic strip of SHR induce more tension by addition of Ca^{2+} than that of KNR, indicating that myoplasmic Ca^{2+} concentration is probably higher in SHR muscle than in KNR. The strong inhibition of contraction induced by the vasoconstrictor may be the reflection of small capacity of intracellular Ca^{2+} store sites in the muscle from SHR. The results of Ca^{2+} influx experiments are consistent with those of tension developments, indicating that the intracellular Ca^{2+} storage capacity of SHR muscle is smaller than that of KNR and that Ca^{2+} uptake is decreased by verapamil more larger in SHR muscle than in KNR. Therefore, the facts observed in the presence experiments strongly suggest that the alteration in membrane systems of vascular smooth muscle may lead to the increase of vascular tone and contribute to the development of hypertension.