40) Changes in platelet function due to hypertension. Defective Ca^{2+} transport and functions in SHRSP platelets. Takako Tomita, Keizo Umegaki, Eiichi Hayashi. Department of Pharmacology, Shizuoka College of Pharmaceutical Sciences, 2-2-1 Oshika, Shizuoka 422.

We have shown that thrombin-, collagen-, ADP- and Ionophore A23187-induced aggregation as well as thrombin-induced serotonin release in washed platelets from SHRSP were greatly reduced with the development of hypertension in comparison with the magnitude of aggregation and release in normotensive WKY platelets (Tomita et al. Stroke 15, 70, 1984). Despite the severe hypo-aggregability observed in SHRSP at 10 - 20 weeks of age, there was no decrease in the serotonin and adenine nucleotide contents in platelets of SHRSP less than 20-weeks of age compared with that in platelets of age-matched WKY. Thus, dysfunction of SHRSP platelets at these ages seems not to be the result of the circulation of degranulated platelets as a consequence of vascular injuries due to hypertension (Tomita et al. JPN Heart J. 25, 879, 1984). Since Ca^{2+} plays a key role in the aggregation and secretion systems, the present study was undertaken to examine Ca^{2+} dependence of platelet functions and Ca^{2+} transport in SHRSP and WKY platelets.

Preparation of washed platelets and measurements of aggregation and malondialdehyde (MDA) were described elsewhere (Tomita et al. J. Pharmacol. Methods 10, 31, 1983). Changes of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in response to thrombin stimulation were followed by using Quin 2 according to the method of Rink et al. (FEBS LETTER 148, 21, 1982). ^{45}Ca uptake was measured by incubating 0.2 ml of platelet (8×10^8 cells/ml) with 20 μl of thrombin and 10 μl of CaCl_2 (final concentration 1.5 mM) containing 2 μCi of ^{45}Ca . The reaction was terminated by an addition of 0.6 ml of cold EDTA (final 5 mM) buffer. Radioactivity uptaken into platelets was counted after platelets were solubilized in Triton X 100 (5 %).

Thrombin (0.22 U/ml)-induced platelet aggregation as well as blood pressure were measured in 18 - 20 week old male WKY, the offspring of WKY and SHRSP, SHR and SHRSP. There were significant inverse correlations between blood pressure and aggregation ($r = -0.783$) and also between the ratios of heart to body weight and aggregation ($r = -0.744$), indicating a close relation of the platelet hypo-aggregability to spontaneous hypertension. Ca^{2+} -dependence of thrombin-induced aggregation and MDA formation and Ionophore A23187-induced aggregation of the platelets from SHRSP at a hypertensive age (16-weeks), was similar to that of platelets from age-matched WKY. There was no difference in aggregation in Ca^{2+} -free medium between the two strains. The enhancement by Ca^{2+} of both thrombin- and Ionophore A23187-induced aggregation, however, was markedly less in SHRSP than in WKY, whereas their MDA formation was equally enhanced by Ca^{2+} . In addition, thrombin-induced thromboxane B_2 formation in SHRSP platelets was similar to that in WKY platelets in the thrombin concentration range of 0.22 - 0.44 U/ml, and became significantly higher at 0.65 U/ml despite severe hypo-aggregability of SHRSP platelets in all the concentrations examined. $[\text{Ca}^{2+}]_i$ increase in response to thrombin (0.03 - 0.11 U/ml) was significantly delayed in 14-week old SHRSP platelets compared with that of age-matched WKY platelets. Maximum $[\text{Ca}^{2+}]_i$ was observed in 30 sec after the stimulation in WKY platelets whereas at 60 sec in SHRSP platelets. There was no difference in maximum $[\text{Ca}^{2+}]_i$ in response to thrombin 0.03 - 0.12 U/ml. $[\text{Ca}^{2+}]_i$ at resting state, however, was slightly lower in SHRSP platelets than in WKY platelets (111 nM in SHRSP vs 138 nM in WKY). Thrombin (0.13 U/ml)-induced ^{45}Ca uptake also was significantly delayed in SHRSP platelets.

In conclusion, abnormalities of SHRSP platelets at hypertensive ages are due to an impaired function of Ca^{2+} concerned in aggregation and secretion but not in the cyclo-oxygenase pathway. A delay of $[\text{Ca}^{2+}]_i$ increase following thrombin stimulation in SHRSP platelets, especially, might greatly influence the rapid synergistic activation of protein kinase C by transiently increased diacylglycerol derived from PI degradation and Ca^{2+} .