Thrombin-induced changes in intracellular Ca^{2+} -concentration and aggregation in platelets from SHRSP and WKY. Ah-Mee Park, Masahiko Ikeda, Masaki Tabuchi, Hideya Mizuno and Takako Tomita. University of Shizuoka, Graduate School of Health Sciences, 52-1, Yada, Shizuoka 422, Japan.

We have previously reported that thrombin and ionophore A23187-induced aggregation, secretion and 47kDa protein (P47) phosphorylation in platelets from stroke-prone spontaneously hypertensive rats (SHRSP) were markedly reduced in comparison with those from Wistar Kyoto rats (WKY). And Ca^{2+} sensitivity of 1-oleoyl-2-acetyl glycerol-induced aggregation, secretion and P47 phosphorylation was also greatly attenuated in saponin-permeabilized platelets from SHRSP. These results suggest that Ca^{2+} activation of protein kinase C which mediates P47 phosphorylation is defective in SHRSP platelets. To confirm this assumption, intracellular Ca^{2+} -homeostasis was examined in relation to thrombin- and ionomycin-induced platelet aggregation in SHRSP and WKY.

Male SHRSP and WKY at age of 14-15 weeks were used in this study. Washed platelets were loaded with $5\mu\text{M}$ fura-2/AM by 30min-incubation. Thrombin and ionomycin-induced aggregation and change of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in Fura-2 loaded platelets (1×10^8 cells/ml) were simultaneously measured using CAF-110 (Nihonbunkou).

There was no significant difference in basal $[\text{Ca}^{2+}]_i$ between SHRSP and WKY platelets. In the presence of extracellular Ca^{2+} (1.5mM), thrombin-induced increase in $[\text{Ca}^{2+}]_i$ in SHRSP were similar to that in WKY in spite of a marked reduction of platelet aggregation in SHRSP. Ionomycin (20-75nM)-induced platelet aggregation and $[\text{Ca}^{2+}]_i$ increase were significantly reduced in SHRSP compared with WKY. However, the degree of reduction in aggregation was greater than that of $[\text{Ca}^{2+}]_i$; ionomycin-induced aggregation as a function of $[\text{Ca}^{2+}]_i$ in SHRSP sifted towards the right of that in WKY. In the absence of extracellular Ca^{2+} , thrombin (0.1, 0.2U/ml) and ionomycin (100, 200nM) did not induce aggregation in both strains, but increased $[\text{Ca}^{2+}]_i$ without significant difference between SHRSP and WKY. Thrombin (0.1 and 0.25U/ml)-induced formation of inositol-1,4,5-trisphosphate (IP_3) in SHRSP platelets was similar to that in WKY platelets.

These results suggested that binding of thrombin to thrombin-receptors and IP_3 to IP_3 -receptors, and Ca^{2+} content in DTS were not altered in SHRSP platelets. However, Ca^{2+} -sensitivity of aggregation pathway is reduced in SHRSP platelets. Thus, this study confirms the previous conclusion that activation by Ca^{2+} of protein kinase C which plays important roles in platelets aggregation, is impaired in SHRSP platelets.