3) Abnormalities in platelet function in stroke prone spontaneously hypertensive rats (SHRSP). Keizo Umegaki, Chikako Nanjo, Takako Tomita, and Eiichi Hayashi. Department of Pharmacology, Shizuoka College of Pharmaceutical Sciences, Shizuoka 422

Platelet adhesiveness and aggregation have been reported to have increased in patients with hypertension. Although platelets are assumed to play important roles in the incidence of stroke in SHRSP, little is known about the platelet function of SHRSP. In this study, the platelet function of SHRSP was investigated, comparing normotensive Wistar Kyoto Rats (WKY) at a prehypertensive (4 weeks), an early hypertensive (11 weeks), and a late hypertensive (17 weeks) stages, using washed platelets. A number of platelets was measured by Coulter Counter Model SP. Washed platelets were prepared according to the method by P. W. Majerus et al. The consequent changes in response to platelet stimulation with proaggregating substances (thrombin and collagen) were followed by phospholipid degradation (induction stage), by malondialdehyde (MDA) formation (transmission stage) and by aggregation (response stage). The phospholipids 5 min after stimulation were separated on HPTLC, and the intensity of fluorescence was measured by a CAMAG HPTLC scanner. MDA formation which reflects the activity of cyclooxygenase pathway, occurs concomitantly with TXA_2 formation. MDA was assayed according to a thiobarbiturate method. Aggregation of washed platelets either with thrombin (0.11 - 0.44 U/ml) or collagen (4.9 - 84 μ g/ml) in the presence of 1.25 mM Ca^{++} was traced by a NKK hema tracer 1. Average blood pressure of SHRSP at 4, 11 and 17 weeks was 109, 215 and 218 mmHg, respectively. The number of platelets in SHRSP was markedly lower with the development of hypertension than those in WKY; $928 \pm 94(9)$ vs $1071 \pm 236(8)$ at 5 weeks, $908 \pm 67(9)$ vs $481 \pm 39^{**}(7)$ at 11 weeks and $761 \pm 86(7)$ vs $451 \pm 87^{*}(6) \times 10^3$ platelets/ml at 17 weeks. With a decrease in the number of platelets, the protein content (μ g/ 10^8 cells) became significantly greater in SHRSP platelets, indicating an increase in size. There was no difference in the cholesterol content on a basis of μ g/mg protein. Aggregability in response to thrombin (0.11, 0.22, 0.44 U/ml) and collagen (4.9, 9.3, 19 μ g/ml) was markedly low with platelets from 11 and 17 week old SHRSP compared with those of age-matched WKY. In contrast, aggregability of platelets from 4 week old SHRSP showed a tendency to be higher than that from WKY. Aggregation (%) of WKY and SHRSP platelets following thrombin (0.22 U/ml) stimulation was $49 \pm 4(4)$ vs $50 \pm 6(5)$ at 4 weeks, $81 \pm 1(5)$ vs $41 \pm 5^{**}(5)$ at 11 weeks, and $75 \pm 3(7)$ vs $32 \pm 5^{**}(7)$ at 17 weeks, while that following collagen (9.3 μ g/ml) stimulation was $47 \pm 3(5)$ vs $60 \pm 3^{**}(7)$ at 4 weeks, $49 \pm 0.1(5)$ vs $16 \pm 1^{**}(7)$ at 11 weeks and $58 \pm 4(7)$ vs $24 \pm 2^{**}(7)$ at 17 weeks. Degradation of phosphatidyl inositol (PI), phosphatidyl choline (PC), and phosphatidyl ethanolamine (PE) 5 min after thrombin (8.0 U/ml) and collagen (84 μ g/ml) stimulation did not differ between WKY and SHRSP at 5 and 11 weeks; the average of % degradation in response to thrombin at 11 weeks was $42 \pm 2.6(5)$ vs $44 \pm 1.4(6)$ in PI, $14 \pm 1.6(5)$ vs $15 \pm 0.8(6)$ in PC and $9 \pm 1.1(5)$ vs $8 \pm 1.8(6)$ in PE. Degradation of PC and PE in the platelets from 17 weeks old SHRSP even showed an increase. MDA formed 5 min after thrombin (0.89 and 8.0 U/ml) and collagen (17 and 84 μ g/ml) stimulation was measured in platelets of SHRSP and WKY at the three ages. MDA formation in response to collagen was almost the same in SHRSP and WKY through the 3 ages, while that in response to thrombin was even greater in SHRSP. There was a strong correlation between platelet aggregation and MDA formation ($r=0.916$). The regression coefficient of SHRSP was greater than that of WKY, indicating that a greater formation of proaggregation substances such as TXA_2 is needed in SHRSP platelets to produce a certain percentage of aggregation compared with WKY. These results suggest that the impairment of platelet functions in SHRSP appears to reside neither in the receptor for the stimulants nor in the transmission of stimulation but in a decreased response to endogenously formed proaggregators.