Biomembrane Characteristics in Stroke-prone Spontaneously Hypertensive Rats (SHRSP)

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Materials and Methods:
Stroke-prone SHR (SHRSP), stroke-resistant SHR (SHRSP), and normotensive Wistar-Kyoto (WK) rats, more than 10 rats of each strain were observed at the age of 50 days and thereafter. An aliquot of 10 µl of blood, either sampled by decapitation or by small incisions on the tail, applied to the 80mM end of the coil, 0.3 mm in diameter and 3 m in length. After the coils were sealed and incubated at 37°C for 10 min, they were centrifuged by the coil planet centrifugation (1500 rpm, self-rotation 16 rpm) for 10 min at 37°C (CPC Model ST, Sanki Engineering Co Ltd, Kyoto). The applied erythrocytes were driven through osmotic gradient from a higher to a lower osmotic pressure (80-20 mM NaSCN) and were hemolyzed at the portion of coils where they could not stand the osmotic stress. The distribution of hemoglobin in the coils was colorimetrically determined or quantitatively assayed using the collected solution from each portion of the coils which were cut into 15 pieces after the centrifugation (Yamori et al: In “Tocopherol, Oxygen and Biomembranes”, Elsevier, Amsterdam, 1978).

Results and Discussion:
In the process of coil planet centrifugation, erythrocytes from SHRSP at the age of 50 days tended to be hemolyzed at a higher osmotic pessure than those from

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age-matched SHRSR and WK rats. At the age of 3 months, this difference in osmotic fragility became clear and the initial and end points of hemolysis were significantly different between erythrocytes from SHRSP and those from SHRSR or WK rats as shown in the Table.

These results clearly indicated the alteration of erythrocyte membrane in SHRSP by detecting the osmotic fragility demonstrated in the process of the coil planet centrifugation. Such difference in biomembrane characteristics may be one extreme example of the more generalized phenomenon related to hypertension or stroke, because altered ionic transport or permeability were observed in the vascular smooth muscle of SHR (Jones: Circulat Res 35: 563, 1973) and also in erythrocytes (Yamori et al: Jap Heart J 18: 604, 1977, Postnor et al: Clin Sci Mol Med 51: 109s, 1976). It is not certain whether these alterations of biomembrane are related to the increased vascular permeability observed in SHRSP and to their stroke-proneness or not. However, as the coil planet centrifugation is a simple enough method to be applicable to the clinical examination of the characteristics of erythrocyte membrane, there is a possibility that the minute difference in biomembrane characteristics detected by this method may be utilized for detecting genetic disposition of stroke-proneness not only in SHRSP but also in man. Comparative clinical studies on such biomembrane characteristics in the offspring from some selected families, in which the incidence of stroke is very high or nearly zero, will be of utmost importance in establishing the method for the prescience of stroke, the first step toward the prevention of stroke.

Summary:
Biomembrane characteristics in stroke-prone SHR (SHRSP) and stroke-resistant SHR (SHRSR) were examined using erythrocytes as a simplified model. When erythrocytes were exposed to osmotic stress in the process of the migration from a higher to a lower osmotic gradient by the coil planet centrifugation, erythrocytes from SHRSP were hemolyzed at a higher osmotic pressure than those from SHRSR. Such a difference in biomembrane characteristics may be useful for detecting a genetic disposition of stroke.

This study was supported by the Science and Technology Agency of Japanese Government, Ministry of Education, National Institute of Health, USA (Grant HL 17754), Japan Medical Research Foundation, Mitsubishi Foundation, Foundation for Research on Metabolic Diseases, Japan Tabacco and Salt Public Corporation, and Foundation for Research on Adult Diseases.