12) Analysis of relaxation of K-depolarized portal vein from SHRSP and WKY after removal of Ca. Nozomu Kurozumi*, Hiroshi Kamiishi*, Tadashi Tezuka**, Keiichi Shimamura***, Tomoko Shimada*** and Satoru Sunano***. *Dept. of Plastic Surgery, Kinki University School of Medicine, **Dept. of Dermatology, Kinki University School of Medicine, ***Research Institute of Hypertension, Kinki University, Osaka-fu 589

We have reported that the rate of relaxation of K-depolarized portal vein by Ca removal was slower in the preparations from SHRSP than in those from WKY control rat and the relaxation time was effected by the intra- or extracellular Na concentration. These results suggest the involvement of Na in the Ca efflux in both SHRSP and WKY portal vein smooth muscle cell. In this study, we analyzed the time course of relaxations of K-depolarized portal veins after Ca removal estimating the change in the intracellular Ca concentration to search for the causative factors for relaxation difference.

Portal veins from SHRSP and WKY of ages of 3-4 months were used. Strips of portal vein were placed in the organ baths containing modified Tyrode's solution. K contracture was induced by K140mM (Na12mM), K80mM (Na60mM) or K140mM (Na0mM) Tyrode's solutions. Ouabain 10^{-3} M was added to the contracting solution to increase the intracellular Na concentration. Relaxation was induced by Ca removal from respective high-K Tyrode's solution. Contractile and relaxing responses were recorded isometrically with a force-displacement transducer.

The time course of the relaxation caused by Ca removal was analyzed by computer. Component analysis by computer revealed that the time course of the relaxation of K-depolarized portal vein consisted of 2 exponential curves both in preparations from SHRSP and WKY. Significant difference of the slope was observed in the relaxation in K140mM (Na12mM) and K80mM (Na60mM) but not in K140mM (Na0mM) Tyrode solution. The time course of changes in intracellular Ca concentration was estimated from the time course of tension decrease taking the relation between contraction of skinned smooth muscle and Ca concentration into computer program. The computed time course of the change in intracellular Ca concentration also showed 2 exponential curves. The 50% relaxation time which was effected by the change of intra- or extracellular Na concentration seemed to depend mainly on the slope of the 1st component. Intracellular Na concentration of portal vein from SHRSP and WKY was determined before and after incubation in 10^{-3} M ouabain. Intracellular Na concentration for preincubation was 21.9 ± 1.2 mmole/kg weight and 15.0 ± 1.1 mmole/kg weight, and after incubation for 60 min they increased to 7.5 ± 4.9 mmole/kg weight and 84.7 ± 3.7 mole/kg weight, respectively. Thus, involvement of Na in Ca efflux in this phase was suggested. Portal vein from SHRSP and WKY were also examined histologically to investigate the changes in diffusion process of Ca in the muscle layer. After soaking portal veins in relaxing solution for more than 30 min, they were fixed with 10% formalin and stained with H.E. The average values of thickness of the muscle layer of portal veins were obtained by the use of computerized image analyzer and were compared.

Average thickness of the muscle layer of SHRSP and WKY portal veins were 107.7 ± 5.0 μ m and 91.6 ± 3.8 μ m, respectively. The Ca diffusion in the extracellular space has been reported to be a rate-limiting step in the relaxation of Ca-dependent contracture under potassium depolarization. The theoretical value for ratio of 50% relaxation time (SHRSP/WKY) is calculated as 1.38, and actual value of relaxation time ratio in Na0-K140 Ca(-), Na12-K140 Ca(-) and Na60-K80 Ca(-) solution is 1.20, 1.44 and 1.60, respectively. Thus, the greater part of the difference of the relaxation curve between SHRSP and WKY may be explained by the difference in the thickness of muscle layer according to the diffusion rate-limiting theory. However, the change of relaxation time with the change of intra- or extracellular Na concentration could not be explained by the difference of diffusion only. Ca movement through cell membrane, such as Na-Ca exchange, should also be taken into consideration.