Ca⁺⁺ Binding Ability and Release of Sarcoplasmic Reticulum from Arterial Smooth Muscle in Spontaneously Hypertensive Rat

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Number of factors may be involved in the regulation of blood pressure. However, the effects of these factors on blood pressure can be achieved through the contraction and relaxation of the cardiac muscle and/or vascular smooth muscle. The muscle contraction and relaxation would be attributed to the intracellular Ca⁺⁺ concentration. Sarcoplasmic reticulum (SR) plays an important role for regulation of intracellular Ca⁺⁺ concentration, in both heart and vascular smooth muscle. Ca⁺⁺ uptake and ATPase activity of isolated SR from the aorta of spontaneously hypertensive rat (SHR) were measured and were compared with these of normotensive Wistar rat (NR), seeking the mechanism of development of hypertension from the nature of SR.

SHR and NR with matched ages (10 to 20 weeks) were used. SR was prepared by the method of Fitzpatrick et al, successive centrifugation of homogenates of small pieces of arterial tissues in mannitol, histidine, and dithiothreitol. Measurements of Ca⁺⁺ binding, bound, and free Ca⁺⁺, were separated using millipore filter and measured utilizing liquid scintillation counter. The maximum binding and apparent binding constants were estimated by double reciprocal plots of free and bound Ca⁺⁺ to SR. ATPase activity was measured Pi liberation by SR, determined by Lowry's method. For the measurement of Ca⁺⁺ content, the sample was hydrolyzed in perchloric acid and concentrated nitric acid at 120°C. Ca was measured using atomic absorption spectrometer.

The mean arterial blood pressure of NR and SHR, 5 weeks of age, was 105 and 145 mmHg, and 15 weeks of age 128 and 190 mmHg, approximately and respectively. ATP dependant Ca⁺⁺ binding by SR of SHR aorta was lesser degree comparing with that of NR aorta. The maximum binding in the presence of ATP and Mg⁺⁺ was 22.16 ± 1.81 (mean $\pm$ S.D.) nmol/mg protein in NR and 17.28 ± 0.03 in SHR, and apparent binding constant was $2.07 \pm 0.16 \times 10^4 \text{M}^{-1}$ in NR and $2.22 \pm 0.08 \times 10^4 \text{M}^{-1}$ in SHR. The maximum Ca⁺⁺ binding of SR from SHR is significantly lower than that from NR ($p < 0.05$). However, there is no significant differences in

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apparent binding constant between NR and SHR aorta ($p < 0.2$). ATP hydrolysis by SR of NR and SHR aorta was enhanced by Mg^{++} and/or Ca^{++} , but ATPase activity of SR from SHR was always lower than that from NR. The optimum concentration of Mg^{++} or Ca^{++} was approximately 2 mM. Freshly prepared SR contained Ca^{++} approximately $2-3 \times 10^{-8}$ mol/mg in both NR and SHR. However, there was a tendency to increase Ca^{++} content in SR with age, and sample from SHR contained higher Ca^{++} than that from NR prepared simultaneously.

It may be concluded that there are certain differences between SR from SHR and NR aorta, particularly in their ATP dependent Ca^{++} binding ability. Because of reduced Ca^{++} capacity of SR, intracellular free Ca^{++} concentration of SHR aorta tends to be higher than that of normal. This may lead to the increase of vascular resistance leading to the system to hypertension, although ionic permeability of cell membrane must be considered as another factor. However, it is not known whether SR in the small arteries will show such differences between NR and SHR.

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