Tension Development and Ca^{2+} Uptake of Isolated Aorta from Spontaneously Hypertensive Rats

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IT is well known that the contractile activity of vascular smooth muscle, as well as that of skeletal muscle, is regulated by myoplasmic free Ca level which is controlled by the function of membrane system. The contraction of vascular smooth muscle induces narrowing of arterial lumen, contributes high total peripheral resistance to blood flow and leads to increase of blood pressure.

Recently, it has been reported that abnormal function of membranes of vascular smooth muscle cell exists in the hypertensive animal and human beings. In hypertension, it is predicted that the muscle cell might have high myoplasmic Ca level due to several reasons, such as (i) hyperactivity of sympathetic nerve, (ii) increased sensitivity of muscle cell to humoral stimulation, (iii) increased Ca permeability of cell membrane and (iv) reduced capacity of intracellular Ca storage sites.

In this study, we investigated tension development and Ca^{2+} influx of rat aorta by several stimulants and inhibitions by Ca^{2+} -antagonists to clarify the difference of vascular smooth muscle functions between Kyoto Wistar normotensive (KNR) and Spontaneously Hypertensive (SHR)¹ rats.

Aortae isolated from age matched male SHR and KNR from 10 to 14 weeks of ages, were used. The aorta was isolated and cut helically along the circular muscle (3×20 mm). The

specimen was incubated in modified Krebs Ringer (in mM, NaCl 119, KCl 4.9, K_2PO_4 1.18, NaHCO_3 14.9, CaCl_2 1.6, MgSO_4 1.17, sucrose 50, dextrose 5.5, pH 6.8) bubbled with 95% O_2 and 5% CO_2 under a resting tension of 4 g at 37°C for 2 hours before the measurement. Isometric tension development in high K^+ medium, serotonin (5HT) and noradrenaline (NA) under the various conditions were measured utilizing a force transducer (SB-1T), amplifier (RM5) and conventional recorder (EPR-3T).

For Ca influx experiments, small pieces (1.0 cm) of aortic tissues were exposed to a Ringer solution containing ^{45}Ca for 15 min. The sample was treated with 10 mM LaCl_3 ² in HEPES buffer and washed, solubilized (tissue solubilizer) and assayed for radioactivity. In all experiments the sample was treated with Ca free Ringer solution containing 2.5×10^{-6} 5HT twice, followed by washing with Ca free Ringer, prior to the measurements. This procedure removes most of the intracellularly stored Ca^{2+} .

The response of KNR aortic strip induced by high K^+ , 5HT and NA with and without Ca in the medium was investigated. K^+ -induced tension only in the presence of Ca in the medium. On the contrary, 5HT and NA-induced tension in Ca free medium was approximately 2/3 to 1/2 of the tension in normal Ringer. NA-induced tension in normal Ringer consisted of initial phasic (fast component) and slow tonic (slow component) fractions, and the slow component disappeared in Ca-free Ringer.

Aortic strip of KNR and SHR developed tension upon addition of stimulants. When the maximum tension development in each stimulant was taken as 100%, aorta from SHR developed

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always greater tension than that from KNR in low concentration of the stimulants. It should be noted that the SHR aorta developed considerable tension by addition of Ca in the absence of any stimulant. When Ca concentration was increased in the medium under the constant dose of stimulants, the tension increased correspondingly. The strip from SHR developed larger tension in all stimulants than that from KNR in all doses of stimulants.

Verapamil inhibited tension development of aortic strip to various stimulations strongly. Two methods of verapamil treatment were employed: (i) preincubation of the specimen in Ca free Ringer containing verapamil for ten min, followed by the addition of Ca and the stimulant, (ii) incubation in Ca Ringer for ten min followed by subsequent addition of verapamil and the stimulant. The method of (i) gave stronger depression of tension than the other procedure in 5HT and NA induced contraction. The fast component of tension was abolished by the pretreatment of the sample with verapamil while in the second component, there was not much differences between two methods. There was no difference of verapamil effect in K^+ induced contraction by the procedure of treatment. In the presence of verapamil, the tension development by aortic strip from SHR was smaller than that by KNR, i.e., the verapamil treatment reduced the tension of SHR strip much more drastically than that of KNR⁴.

Nifedipine had a stronger inhibitory action on K^+ -induced tension of aortic strips, about 20 times greater than that of verapamil. The effect of nifedipine, as well as that of verapamil, appeared to be greater in aorta from SHR than that from KNR.

Ca influx to the vascular smooth muscle cell in the Ringer solution containing various amount of Ca, and the effect of verapamil were measured. The strip from SHR took less Ca from the medium in high Ca concentration range comparing with that of KNR in both presence and absence of verapamil during the 15 min incubation period. The inhibitory action of verapamil on Ca influx was stronger in SHR than in KNR⁴.

The results obtained in these experiments suggest that the abnormality of the membrane system exist in the aortic smooth muscle in SHR, i.e., increased Ca permeability of cell membrane or reduced capacity of internal Ca storage sites⁵.

In this experiment, the starting materials contained no Ca in the internal storage sites. The

fact that the aortic strips of SHR in Ca free medium developed more tension by the addition of Ca indicates that myoplasmic free Ca concentration is higher in SHR muscle than that in KNR in normal condition. The elevated myoplasmic Ca level in SHR vascular muscle may be caused by the abnormality of the membrane system. The strong inhibition of NA-induced tension in SHR aortic strip by verapamil or nifedipine would reflect the small capacity of intracellular Ca storage sites in SHR comparing with those in KNR.

The results of Ca influx experiments and inhibition by verapamil are consistent with those of tension measurements indicating the intracellular Ca storage is less in the vascular muscle of SHR. Ca entered into the cell is quickly absorbed into the storage sites or extruded to the outside of the cell. So, if the membrane system is working, free Ca in the cell should be kept quite low level.

We have previously shown that isolated sarcoplasmic reticulum from SHR aortic muscle exhibited a reduced Ca uptake⁵. Also, Jones⁶ has shown that increased permeability of Ca through the cell membrane of SHR vascular smooth muscle. Together with these reports, the results obtained in the present experiment strongly suggest that alteration of membrane of vascular smooth muscle may be one of the causes to increase vascular tone leading to the development of hypertension.

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