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Difference of the inhibitory action of verapamil on the positive inotropic effect of Ca^{2+} between spontaneously hypertensive and normotensive rat myocardium

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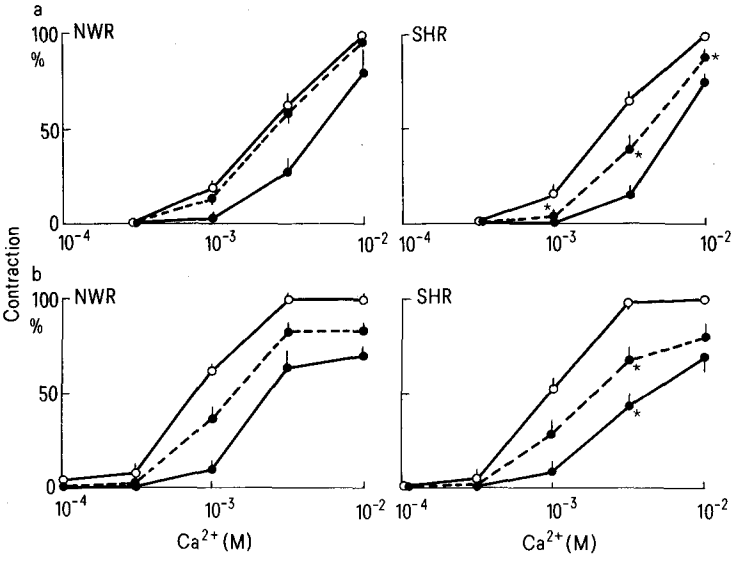
Summary. Verapamil, a calcium entry blocker, had a greater inhibitory effect on the positive inotropic effect of excess Ca^{2+} in SHR than in NWR, suggesting that the cardiac responsiveness to verapamil was enhanced in SHR.
Key words. Rat myocardium; myocardium, rat; hypertensive rats, spontaneously; atrium; papillary muscle; verapamil; inotropic effect, calcium.

The differences between spontaneously hypertensive rats (SHR) and normotensive Wister rats (NWR) in the handling of calcium for contraction in vascular smooth muscle have previously been demonstrated. Calcium channel inhibitors, such as verapamil and nifedipine, had a greater inhibitory effect on the aortic contraction of SHR than on that of NWR[2]. Pederson et al[3] also found that in the SHR, excess calcium induced contraction of the aorta, which was more sensitive to nifedipine than the response in the NWR, suggesting a larger dependency of extracellular Ca^{2+} in the SHR vascular contraction. In fact, in patients with essential hypertension intra-arterial perfusion of verapamil and nifedipine caused a marked vasodilation, suggesting a functional abnormality in essential hypertension with increased dependency of arteriolar tone on calcium influx[4-6]. These findings demonstrate that the vascular smooth muscle in hypertension had an abnormality in calcium flux. Although such an abnormality in the vascular system is an important factor in the development of hypertension, changes in cardiac responsiveness are also involved in the disease. However, myocardial responsiveness of SHR to calcium entry blockers has not been reported yet. In the present study, the influence of verapamil on the positive inotropic effect of excess Ca in the isolated atrial and papillary muscles from SHR were compared to that in the preparations from NWR.

Materials and methods. Adult spontaneously hypertensive male Wister rats of the Kyoto strain (SHR) developed by Okamoto and Aoki[7], and normotensive Wistar rats (NWR) 3–5 months old were used. The blood pressure for SHR and NWR measured by the tail-plethysmographic method was 173 ± 1 mm Hg ($n = 10$) and 127 ± 3 mm Hg ($n = 10$), respectively. The animals were killed by a blow on the head. The hearts were quickly excised and dissected in Krebs-Ringer solution. The papillary muscles from the right ventricle and the left atrium were isolated. Each preparation was mounted in a tissue bath containing 20 ml of Krebs-Ringer solution of the following



The effect of verapamil (10^{-7} M: ●----● and 10^{-6} M: ●——●) on contraction induced by cumulative addition of calcium of atria (a) and papillary muscles (b) from NWR and SHR. Contraction is represented as a percent of the maximum contraction of control. Vertical lines indicate the mean ± SE of 5 preparations. *Values are significantly different from those of NWR ($p < 0.05$).

composition (in mM): NaCl, 120.3; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4 \cdot 7H_2O$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 24.2; and glucose, 5.5 (pH 7.4). The temperature was maintained at 30 °C, and Krebs-Ringer solution was oxygenated with a mixture of 95% O_2 and 5% CO_2. The preparations were connected to a force-displacement transducer for recording isometric tension. The resting tension applied to papillary muscles and atria was adjusted to 0.2 g and 0.8 g, respectively. The preparations were constantly stimulated at 1.0 Hz and equilibrated for 90 min before experiments. Contractions were induced by cumulative addition of calcium (0.3, 1, 3, 10 mM) after exposure to Krebs-Ringer solution which contained 0.1 mM Ca^{2+} and no KH_2PO_4 for 20 min. Verapamil was added when the normal solution was changed to modified solution. ED_{50} and ID_{50} represents the calcium concentration to induce 50% of maximal contraction and the concentration of verapamil to induce a 50% inhibition of contraction, respectively. Statistical analysis was performed using Student's t-test, and significance was established at $p < 0.05$.

Results and discussion. In atrial and papillary muscles of both SHR and NWR, cumulative addition of Ca^{2+} (10^{-4} M–10^{-2} M) caused a positive inotropic effect in a concentration-dependant manner (fig.). ED_{50} for Ca^{2+} in these preparations are as follows: $2.2 \pm 0.2 \times 10^{-3}$ M in SHR atria, $2.3 \pm 0.3 \times 10^{-3}$ M in NWR atria, $1.0 \pm 0.1 \times 10^{-3}$ M in SHR papillary muscle and $0.8 \pm 0.05 \times 10^{-3}$ M in NWR papillary muscle (n = 5). The treatment with verapamil at 10^{-7} M significantly shifted the concentration response curve of Ca^{2+} to the right from the untreated control response curve in SHR atria but had no effect on the response curve in NWR atria (fig., a). At a higher concentration (10^{-6} M), however, even in NWR atria, verapamil shifted the response-curve of Ca^{2+} to the right from the control curve. Also, this higher concentration further decreased the positive inotropc effect of Ca^{2+} in SHR atria. The potency of verapamil as an inhibitor of 1 mM Ca^{2+}-induced contraction (ID_{50}) was greater in the SHR atria ($0.3 \pm 0.2 \times 10^{-7}$ M, n = 5) than in the NWR ($2.5 \pm 0.4 \times 10^{-7}$ M, n = 5).

These results indicate an increased sensitivity to verapamil in the SHR atria. Pederson et al.[3] have reported a similar phenomenon in the inhibitory effect of nifedipine, another calcium antagonist, on the contractile response of the SHR thoracic aorta. To our knowledge, such a demonstration on cardiac muscle has not been published, although the altered reactivity of SHR atria to β-receptor stimulant has previously been demonstrated by Fujiwara et al.[8]. On the other hand, in papillary muscles of both SHR and NWR (fig., b), a lower concentration of verapamil, 10^{-7} M, inhibited the positive inotropic effect of Ca^{2+}. A higher concentration of verapamil (10^{-6} M) caused further decrease in the contraction in both the SHR and the NWR papillary muscles. The inhibitory action of verapamil on the contractile responses of papillary muscle to Ca^{2+} at 0.3, 1.0 and 10 mM was not different between SHR and NWR. Only the contraction induced by 3 mM Ca^{2+} was inhibited more effectively in the SHR than in the NWR preparations by verapamil. ID_{50} of verapamil in 1 mM Ca^{2+}-induced contraction of papillary muscle was $1.4 \pm 0.6 \times 10^{-7}$ M in the SHR and $2.1 \pm 0.8 \times 10^{-7}$ M in the NWR. The increased sensitivity of the SHR myocardium to verapamil was less in papillary muscles than in atria. This difference may possibly be related to the different sensitivity to calcium in myocardium shown in the present data for ED_{50} values.

The present study demonstrated that the inotropic response of the working myocardium of SHR was more sensitive to verapamil than that of NWR, although in papillary muscles such increased sensitivity was apparent only in a narrow calcium concentration range. This suggests that the properties of the slow channel of SHR myocardium may be different from that of NWR. Differences in calcium kinetics through the cardiac cell membrane which may exist between SHR and NWR can perhaps be considered to be an important factor in the development of hypertension, as well as the abnormal calcium kinetics in the SHR vascular smooth muscle reported by Zsoter et al.[9].

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