Enhanced Vasorelaxation Response to Cromakalim in Spontaneously Hypertensive Rats

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= ABSTRACT =

To investigate the properties of cromakalim-opened K^+ channels in aorta of spontaneously hypertensive rats (SHR), the effect of cromakalim on tension was compared in endothelium-rubbed aortic rings from SHR and normotensive Wistar-Kyoto rats (WKY).

1. Cromakalim relaxed the aortic ring contracted by 10^{-7} M norepinephrine (NE) dose-dependently, and this relaxant response to cromakalim was blocked by 10^{-5} M glybenclamide.

2. Cromakalim also relaxed the contraction induced by high K^+-solution or 10 mM tetraethylammonium dose-dependently. However, the relaxant response to cromakalim was decreased by raising the K^+ concentration.

3. SHR aorta exhibited myogenic tone in resting state which was inhibited by cromakalim, verapamil or Ca^{2+}-free PSS. Whereas, WKY aorta did not exhibit any myogenic tone in resting state.

4. When aortic rings from both strains were contracted by 20 mM K^+ or NE, relaxant responses to low concentration of cromakalim (below 10^{-7} M) were not different between WKY and SHR, but maximum relaxant response to cromakalim (above 3×10^{-7} M) was greater in SHR than in WKY.

5. When the relaxant response to cromakalim was expressed as percent of maximum relaxation induced by Ca^{2+}-free PSS, relaxant response to cromakalim in 20 mM K^+-induced contraction was not different between WKY and SHR.

From the above result, it is suggested that relaxant responses to cromakalim are greater in SHR than WKY, and this may be due to the myogenic tone of aortic rings from SHR.

Key Words: Aorta, Cromakalim, Glybenclamide, K^+-channel, Hypertension

INTRODUCTION

Cromakalim is a vasodilator known as a potassium channel opener. Considerable evidence suggests that the specific site of action of potassium channel openers such as cromakalim, pinacidil and calcitonin gene related peptide is the ATP-sensitive K^+-channel (Standen et al, 1989). This type of K^+-channel is characterized by a pronounced reduction in channel activity by cytosolic ATP (Cook & Hales, 1984; Quast & Cook, 1989).

The blood pressure-lowering and anti-vasoconstrictor effects of cromakalim are well shown in spontaneously hypertensive rats (SHR) (Buckingham, 1988; Struijker Boudier et al, 1992). SHR have been reported to be more sensitive to the blood pressure-lowering effect of the cromakalim than

normotensive Wistar-Kyoto rats (WKY) (Asano et al, 1994; Falotico et al, 1989; Furspan & Webb, 1993; Miyata et al, 1990), but the underlying mechanism has not been clearly elucidated. Furspan and Webb (1993) reported that the ATP sensitivity of K_{ATP} was decreased and relaxant response to cromakalim was enhanced in vascular smooth muscle from genetically hypertensive rats. However, Asano et al (1994) reported that increased basal Ca^{2+} influx and a high activation of Ca^{2+}-activated K^+-channel (K_{Ca}) in resting state of SHR arteries were the causes of greater response to cromakalim in SHR than in WKY.

The purpose of present study was to evaluate the effect of cromakalim on the mechanical response of isolated aortic rings and to compare the effects of cromakalim on the tension in aortas from WKY and SHR.

METHOD

Preparation of aortic segment

SHR, 18～22 weeks of age, and age-matched WKY were used. The systolic blood pressure at this age, measured by tail-cuff plethysmographic method, of SHR and WKY was 202 ± 6 mmHg and 131 ± 10 mmHg, respectively.

The rats were stunned by blow to the head and exsanguinated. The thoracic aortas were excised and placed in a physiological salt solution (PSS) of the following composition (mM) : NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5, glucose 5 (pH was adjusted to 7.4 with NaOH.). The aortas were cleaned of connective tissue and allowed to recover for 2 hours at room temperature. The aortas were removed of endothelial cell by gentle rubbing by cotton wool and cut into ring segments (2～3 mm wide).

Measurement of isometric tension

Aortic rings from SHR and WKY were mounted in a organ bath (50 ml) containing a PSS. The

solution was maintained at $37^\circ C$ and aerated with 100% O_2. The isometric tension was recorded with a force-displacement transducer (F-60 Narco-Bio system). Rings from SHR and WKY were stretched passively to optimal length by imposing a resting tension of 2 g and a 90-min equilibrium period preceded each experiment.

After equilibration, contractile responses of the aortic rings to PSS containing 60 mM KCl (K^+ substitution for Na^+) were repeated twice or three times until the resposes were reproducible. After washout relaxant effects of cromakalim were determined in 20 mM K^+- or norepinephrine-contracted aortic rings. Concentration-response curves for the relaxant effects of cromakalim were constructed by the cumulative addition of agents. In some experiments, 10^{-6} M sodium nitroprusside (SNP) was added at the end of experiment to identify the position of the maximum relaxation. Relaxant responses to cromakalim and sodium nitroprusside are expressed as % of the K^+- or norepinephrine-induced contraction or expressed as % of maximum relaxation induced by Ca^{2+}-free PSS.

Statistical analysis

The EC_{50} value for cromakalim was obtained from a plot of % responses vs. log concentration of cromakalim and expressed as a negative log (pD_2 value). Unless specified, the results are expressed as means $\pm$ S.E.M. (n=number of preparation). The Student's t-test for paired data was used to determine the significance of differences between means, and a P value of < 0.05 was taken as significance.

Drugs

The drugs used were cromakalim (Biomol), glybenclamide (Sigma), tetraethylammonium (Sigma), sodium nitroprusside (Sigma), l-norepinephrine bitartrate (Sigma). Cromakalim (10 mM) was dissolved in 50% ethanol and 50% polyethylene glycol with further dilution in PSS before use.

Glybenclamide (10 mM) was dissolved 100% DMSO with further dilution in PSS before use.

RESULTS

Relaxation of norepinephrine-contracted aortic rings by cromakalim

The relaxant effects of cromakalim were determined in norepinephrine-contracted aortic rings (Fig. 1A). The cumulative addition of cromakalim to the norepinephrine-contracted state of WKY aorta caused a concentration-dependent relaxation. However, norepinephrine-contracted aortic rings did not relaxed completely by cromakalim. The effect of glybenclamide, a blocker of ATP-sensitive potassium channel, on the relaxant responses to the

Fig. 1. Typical recordings show the effect of cromakalim on norepinephrine (NE)-induced contraction (A), and show the effect of glybenclamide (10⁻⁵ M) on cromakalim-induced relaxation (B) in aortic rings from WKY. Concentrations of cromakalim are expressed as a negative log of the molar concentration.

cromakalim were determined in norepinephrine-contracted aortic ring (Fig. 1B). Glybenclamide (10^{-5} M) was added 40 minutes before the norepinephrine-induced contraction. Pretreatment with glybenclamide inhibited the relaxation response to cromakalim in WKY aorta.

Relaxation of TEA- or K+-contracted aortic rings by cromakalim

Effects of TEA were compared in resting state of WKY and SHR aorta (Fig. 2A). Cumulative concentrations (0.1 ~ 10 mM) of TEA were applied to block the basal K^+ conductance in WKY and SHR aorta. In all experiments(n=6), application of TEA resulted in a large dose-dependent contraction of SHR aorta, whereas WKY aorta showed contractile response in only 3 out of 10 preparations. The magnitude of maximum contractions were $24.8 \pm 8.1\%$ in WKY and $72.9 \pm 7.7\%$ in SHR as compared with 60 mM K^+-induced contraction, respectively. Under these contractions, cromakalim caused a concentration-dependent relaxation and these relaxant responses to cromakalim were inhibited by glybenclamide pretreatment (Fig 2B).

Effects of cromakalim were determined in K^+-contracted WKY aorta (Fig 3). In the PSS containing 20, 35 or 60 mM K^+, WKY aorta showed sustained contraction. The addition of cromakalim to these aortas caused a concentration-dependent relaxation in PSS containing 20 or 35 mM K^+, whereas WKY aorta in 60 mM K^+ solution did not relax by cromakalim. The maximum relaxations of WKY aortas by cromakalim were 100 and $43.6 \pm 5.9\%$ in 20 mM K^+ and 35 mM K^+ solution, respectively.

Relaxation of resting aorta by cromakalim

Relaxant effects of cromakalim were determined in resting aortas from WKY and SHR (Fig. 4). SHR aorta maintained a myogenic tone in resting state. The addition of cromakalim to the resting state of

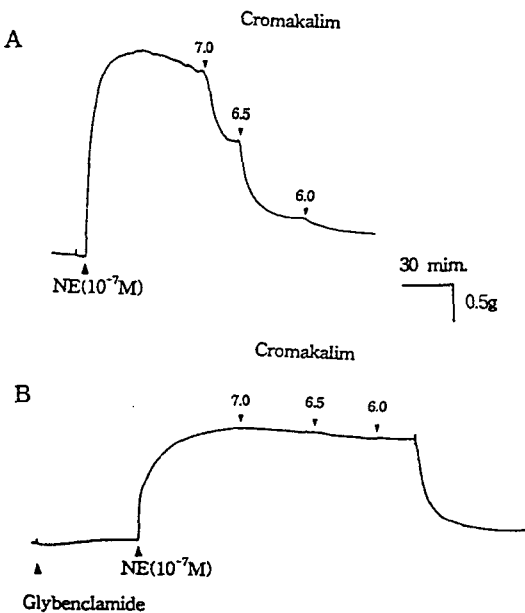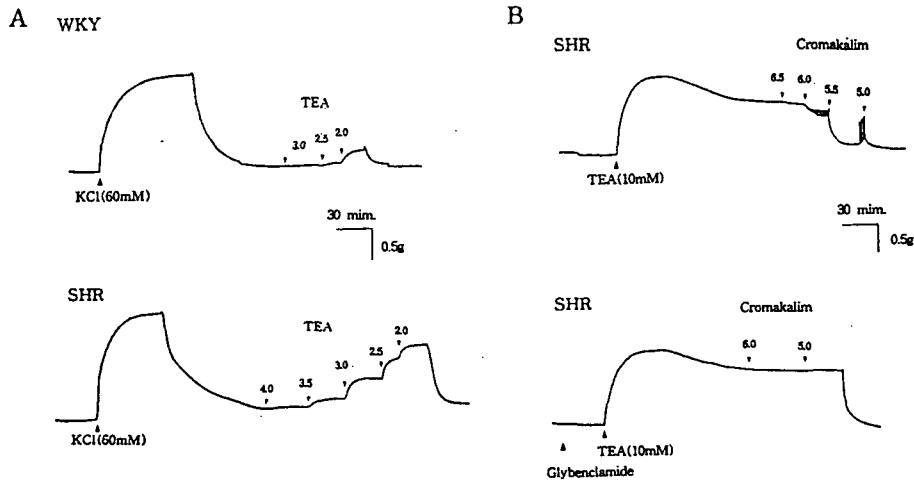A   WKY

SHR

B

SHR   Cromakalim

SHR   Cromakalim

Fig. 2. (A) Effects of tetraethylammonium (TEA) on aortic rings from WKY and SHR. Aortic ring from WKY exhibited less contractile response to TEA than that from SHR. (B) Effect of glybenclamide on cromakalim-induced relaxation in aortic ring from SHR. Contraction induced by TEA (10 mM) was inhibited dose-dependently by cromakalim and cromakalim-induced relaxation was blocked by pretreatment of glybenclamide (10^{-5} M) completely. Concentrations of TEA and cromakalim are expressed as a negative log of the molar concentration.
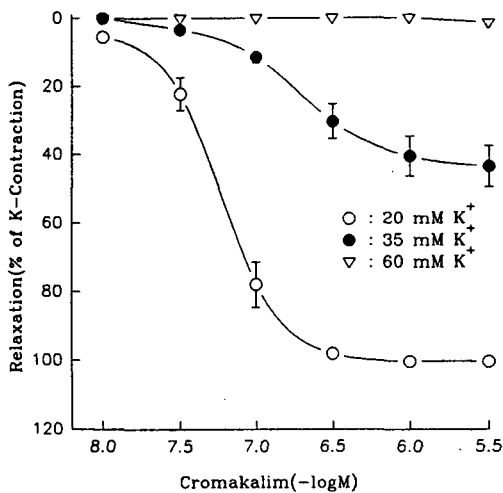

Fig. 3. Concentration-response curves for relaxant response to cromakalim in K^+-induced contraction of aortic rings from WKY. After 2-hour equilibration, aortic rings were contracted by 20 mM K^+, 35 mM K^+ or 60 mM K^+ respectively. When the contraction reached a plateau, cromakalim was added in a cumulative fashion. Cromakalim-induced relaxation are expressed as % of maximal contraction induced by each K^+ concentration. Data points are means from five preparations and S.E.M. are shown by vertical bars.
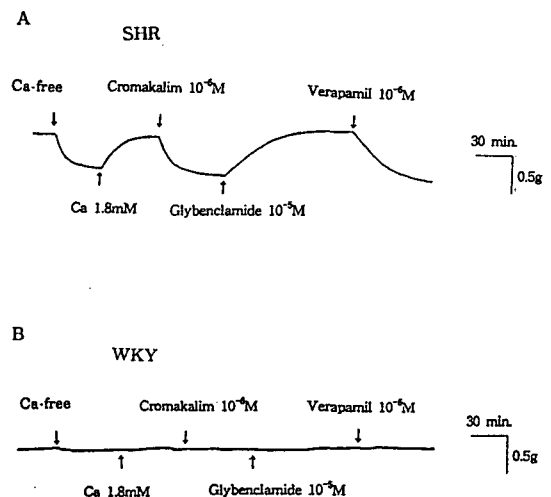
A   SHR

B   WKY

Fig. 4. Typical recordings of the effects of extracellular Ca^{2+}, cromakalim and verapamil on resting strips aortas from SHR and WKY. (A) Aorta from SHR exhibited myogenic tone in resting state, and the myogenic tone was suppressed by removal of extacellular Ca^{2+}, addition of cromakalim or verapamil. (B) Aorta from WKY did not exhibit any myogenic tone in resting state.

SHR aorta caused relaxation from resting tone of aorta and addition of glybenclamide completely restored myogenic tone. The addition of verapamil also caused relaxation from resting tone of aorta. The SHR aorta relaxed significantely from the resting tone when placed into a Ca^{2+}-free solution. After exposure to the Ca^{2+}-free solution, the addition of 1.8 mM Ca^{2+} completely restored the myogenic tone. However, myogenic tone was not detected in WKY aorta. The WKY aorta did not show a relaxation in response to cromakalim, verapamil or Ca^{2+}-free solution.

Different relaxant response to cromakalim in WKY and SHR aorta

The relaxant effects of cromakalim were compared in norepinephrine-contracted aortas from WKY and SHR (Fig. 5). The addition of cromakalim to aortas, contracted by norepinephrine, caused a concentration-dependent relaxation. The relaxant responses to relatively high concentrations of cromakalim were greater in SHR ($105\pm3.9\%$) than in WKY ($80.4\pm3.7\%$). However, the pD_2 value for cromakalim was not significantly different between SHR (6.79 ± 0.68, n=5)) and WKY (7.05 ± 0.13, n=5).

The relaxant effects of cromakalim were also compared in K^+-contracted aortas from WKY and SHR (Fig. 6). In PSS solution containing 20 mM K^+, the aortas showed a sustained contraction. The addition of cromakalim to these aortas caused a concentration-dependent relaxation. Although the maximum relaxation induced by relatively high concentration of cromakalim was significantly
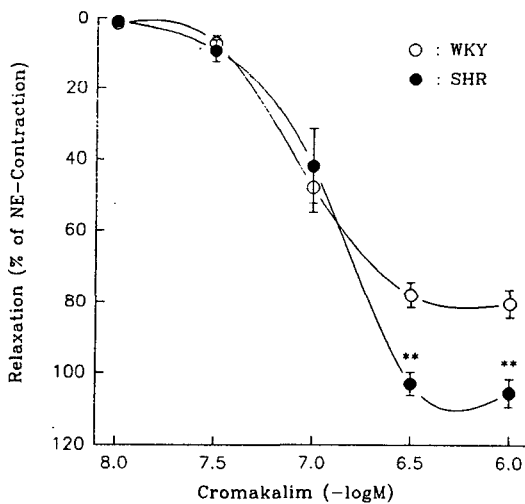

Fig. 5. Relaxant effect of cromakalim on NE-induced contraction of aortas from WKY and SHR. After 2 hour equilibration, the aortas were contracted by NE and cromakalim was added in a cumulative fashion. Cromakalim-induced relaxation are expressed as % of the contraction induced by NE. Data points are means from five preparations, and S.E.M. are shown by vertical bars. ** Significantly different from WKY (p<0.05)
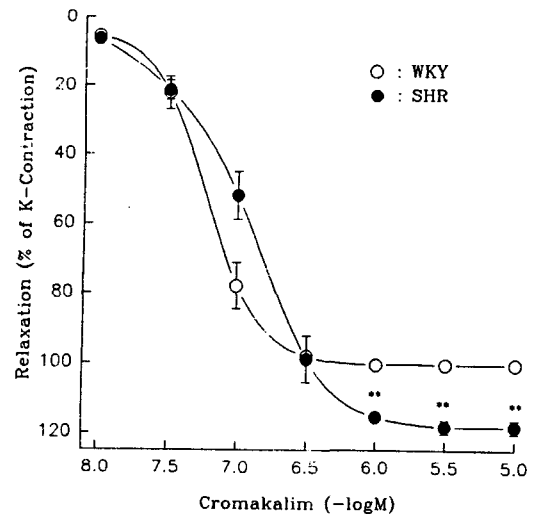
Fig. 6. Relaxant effect of cromakalim on K-induced contraction of aortas from WKY and SHR. After 2 hour equilibration, the aortic ring was contracted by 20 mM K^+, and cromakalim was added in a cumulative fashion. Cromakalim-induced relaxations are expressed as % of maximal contraction induced by 20 mM K^+. Data points are means from 5 preparations, and S.E.M. are shown by vertical bars. ** Significantly different from WKY (p<0.05)
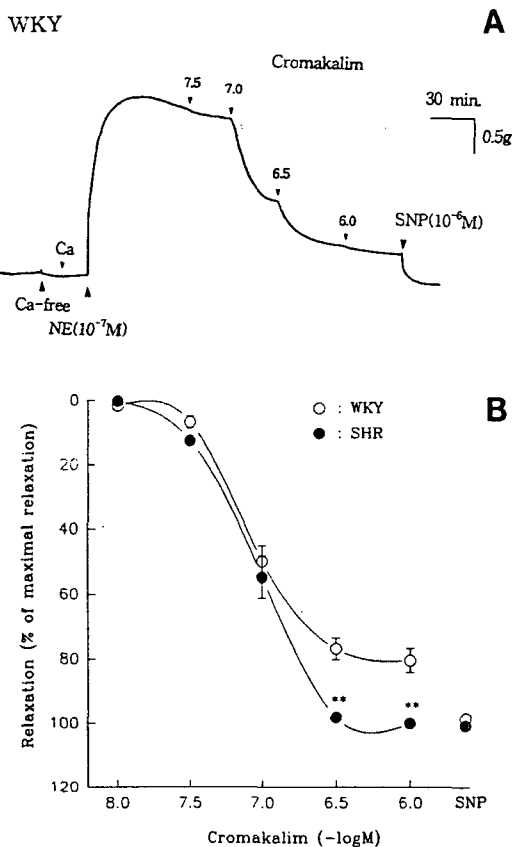
Fig. 7. Relaxant effect of cromakalim on NE-induced contraction of aortas from WKY and SHR. (A) After 2 hour equilibration, aortas were exposed to Ca^{2+}-free solution until the myogenic tone was supressed, and Ca^{2+} 1.8 mM was readded. After the amplitude of myogenic tone was confirmed, NE was applicated and cromakalim was added in a cumulative fashion. At the end of each experiment, 10^{-6} M SNP was added to identify the maximum relaxation. Concentrations of cromakalim and SNP are expressed as a negative log of the molar concentration. (B) Cromakalim- and SNP-induced relaxations are expressed as % of the maximal relaxation induced by Ca^{2+}-free PSS. Data points are means from five preparations, and S.E.M. are shown by vertical bars. ** Significantly different from WKY (p<0.05)
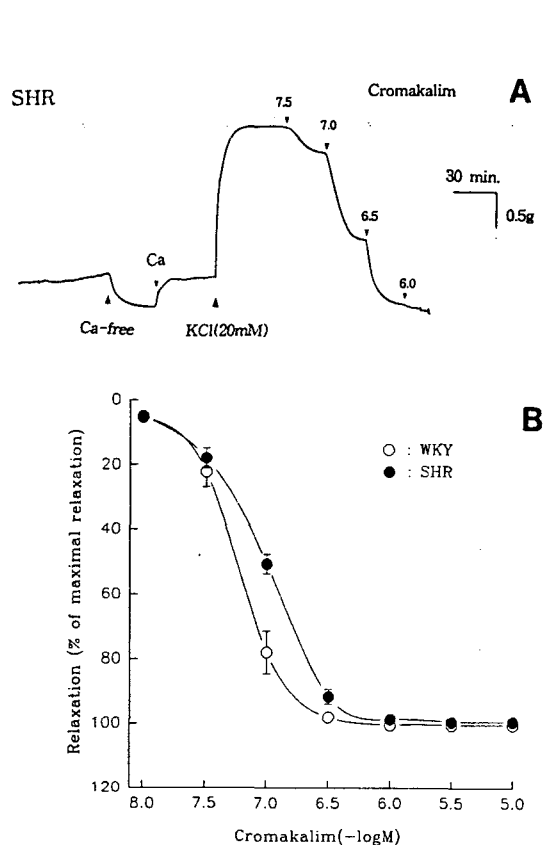
Fig. 8. Relaxant effect of cromakalim on K-induced contraction of aortas from WKY and SHR. (A) After 2 hour-equilibration, aortas were exposed to Ca^{2+}-free solution until the myogenic tone was suppressed, and Ca^{2+} 1.8 mM was readded. After the amplitude of myogenic tone was confirmed, PSS was replaced by 20 mM K^{+}-solution and cromakalim was added in a cumulative fashion. Concentrations of cromakalim are expressed as a negative log of molar concentration. (B) Cromakalim induced relaxations are expressed as % of the maximum relaxation induced by Ca^{2+}-free PSS. Data points are means from five preparations, and S.E.M. are shown by vertical bars.

greater in SHR (118.3±1.9%) than in WKY (100.5 ±0.6%), the pD2 value for cromakalim was not significantly different between SHR (6.88±0.67)

and WKY (7.28±0.07).

It is noteworthy that aortas from SHR exhibited myogenic tone in the resting state and the position of the SNP- or cromakalim-induced relaxation was far below the resting tone. Therefore, the relaxations

induced by each concentration of cromakalim were expressed as % of the maximum relaxation induced by Ca^{2+}-free PSS. In 20 mM K-induced contraction, there is no significant difference in the maximum relaxation induced by relatively high concentration of cromakalim between SHR and WKY (Fig. 8). However, in norepinephrine-contracted aorta, cromakalim-induced maximum relaxation was greater in SHR (100%) than in WKY ($80.4 \pm 3.7\%$) (Fig. 7).

DISCUSSION

The membrane potential of arterial smooth muscle cells, which is regulated by K^+ channels, is an important regulator of arterial tone and hence arterial diameter. The opening of K^+ channels in the cell membranes in arteries increases K^+ efflux, which causes membrane potential hyperpolarization. This closes voltage-dependent Ca^{2+} channels, decreasing Ca^{2+} entry, which leads to vasodilatation. Thus defects in K^+ channel function may lead to vasoconstriction or vasospasm as well as compromise the ability of an artery to dilate. Alteration in smooth muscle K^+ channel function may be involved in pathological conditions of the vasculature such as vasospasm, hypertension, ischemia, hypotension during endotoxic shock (Landry & Oliver, 1992), and changed vascular reactivity during diabetes. Four distinct types of K^+ channels have been identified in arterial smooth muscle : 1) voltage-dependent K^+ (Kv) channels, 2) Ca^{2+}-activated K^+ (K_{Ca}) channels, 3) inward rectifier K^+ (K_{IR}) channels, and 4) ATP-sensitive K^+ (K_{ATP}) channels. Modulation and expression of these channels vary with vascular bed and between larger arteries and resistance arteries (Nelson & Quayle, 1995).

Cromakalim, a benzopyrene derivative, is one of several antihypertensive agents that are believed to cause relaxation of smooth muscle by opening K^+ channels (Hamilton et al, 1986; Weir & Weston, 1986; Quast, 1987). Cromakalim has been shown to

directly activate K_{ATP} channels in vascular smooth muscle (Brayden et al, 1991; Standen et al, 1989; Winquist et al, 1989). A few studies indicated that cromakalim activated K_{Ca} channels (Gelband et al, 1989; Hu et al, 1990; Stockbridge et al, 1991) or directly inhibited Ca^{2+} channels (Leblanc et al, 1989; Okabe et al, 1990) in vascular smooth muscle. In our study, cromakalim inhibited NE-induced contractions as well as K^+ (20~35 mM)-induced contractions. Under these conditions, the equilibrium potential for K^+ (E_k), would be more negative than the normal resting membrane potential. Therefore, K^+-channel activation by cromakalim would increase K^+ conductance and hyperpolarize the vascular smooth muscle sufficiently to inhibit Ca^{2+} entry. However, cromakalim did not inhibit contractions at high K^+ concentrations (60 mM K^+). Under these conditions, increase in K^+ conductance by cromakalim would not hyperpolarize vascular smooth muscle cell sufficiently to inhibit Ca^{2+} entry, presumably because E_k would be less negative than the potential to close voltage-dependent Ca^{2+} channels. We also observed the effect of glybenclamide, a well known inhibitor of K_{ATP} channels in a variety of tissues (Ashcroft & Ashcroft, 1990; Buckingham et al, 1989; Cavero et al, 1992; Noma, 1983). At 10 uM, glybenclamide inhibited the relaxing effect of cromakalim completely. In contrast, TEA, which is known to block large-conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle, failed to block the cromakalim-induced relaxation. Above results indicate that cromakalim causes vasorelaxation via activation of K_{ATP} channel not via activation of K_{Ca} channel or inhibition of Ca^{2+} channels.

SHR arteries have been shown to maintain a myogenic tone in resting state (Noon et al, 1978; Winquist & Bohr, 1983; Asano et al, 1993). In the present study, SHR aorta also exhibited myogenic tone in the resting state. The myogenic tone was not affected by glybenclamide but abolished by cromakalim, suggesting that the cromakalim-activated

K^+ channels are present but do not appear to be activated in association with the myogenic tone in SHR aortas. However, in WKY aorta, cromakalim failed to cause a relaxation from the resting tone, suggesting that these aortas are already completely relaxed. The myogenic tone was also abolished by verapamil or Ca^{2+}-free PSS in SHR aorta. Above results suggest that the myogenic tone is due to the increased Ca^{2+} influx through voltage dependent Ca^{2+} channels. The increased cellular Ca^{2+} uptake or content in the resting state has been demonstrated in aorta from SHR (van Breemen et al, 1986; Jelicks & Gupta, 1990; Sada et al, 1990) and membrane depolarization in SHR arteries has been demon-strated in the mesenteric artery (Fujii et al, 1992), tail artery (Cheung, 1984) and aorta (Tomobe et al, 1991). Increased cellular Ca^{2+} uptake or membrane depolarization leads to activation of K_{Ca} channels in vascular smooth muscle because the K_{Ca} channels are activated by both cellular Ca^{2+} and membrane depolarization. The present study clearly demonstrated that the TEA-sensitive K^+-channels were highly activated in the resting state of aortas from SHR when compared to WKY. High activation of K_{Ca} channels in the resting state of SHR aortas may reflect the membrane depolarization and increased cellular Ca^{2+} uptake.

Present study demonstrated that cromakalim-induced maximal relaxation was greater in SHR aorta when compared to WKY aorta. In 20 mM K^+- or norepinephrine-contracted arteries, SHR exhibited an increased maximum relaxation and its maximum relaxant response is more than 100% when ex-pressed as % of 20 mM K^+- or norepinephrine-induced contraction. Whereas, maximum relaxant response to cromakalim in WKY aorta is less than 100%. However, concentration-relaxation curve for cromakalim was not shifted to left in SHR when compared to WKY and pD_2 value for cromakalim was not significantly different between SHR and WKY. The results suggest that an increased relaxant effect of cromakalim in SHR is not due to the

altered sensitivity of K_{ATP} channels to cromakalim. Furspan and Webb (1993) reported that although the effects of diazoxide and glybride on contractile responses were different in SHRSP and WKY rats, the effects were not different at the single channel level. When the relaxations induced by each con-centration of cromakalim are expressed as % of the maximum relaxation induced by Ca^{2+}-free PSS, there is no significant difference in maximum relaxation between SHR and WKY. Above results suggest that the increased maximal relaxant response to cromakalim in SHR may be due to the myogenic tone in resting state. SHR aorta exhibited myogenic tone which is inhibited by cromakalim or verapamil. Therefore, in the 20 mM K^+- or norepin-ephrine-contracted SHR aorta, cromakalim could relax aorta more than 100% and exhibited increased maximum relaxation when compared to WKY aorta. Whereas, WKY aorta did not exhibit any myogenic tone in resting state. Thus cromakalim could relax aorta no more than 100% in WKY

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