Chloride Channel Activity of Vascular Smooth Muscle in the Spontaneous Hypertensive Rats

Zhen Wang[1], Qiang Chai[2], Zhixiang Liu[2], Dexiang Liu[3], and Lianbi Chen[1]*

[1]*Institute of Physiology, School of Medicine*
Shandong University
[2]*Department of Physiology*
Shandong Academy of Medical Sciences
[3]*Department of Medical Psychology, Shandong University*
Jinan, Shandong Province, 250000
People's Republic of China

Abstract

To characterize the activity of the Ca^{2+}-activated Cl^- channels in vascular smooth muscle (VSM) of the spontaneous hypertensive rats (SHR), the isolated mesenteric vascular beds and tail artery strips were preparated from SHR and Wistar rats aged 7-8 weeks. The changes in contractile response to norepinphrine (NE) were taken as an index of vascular mortion. Results showed that the contractile responses of mesenteric arteries and tail arteries to NE in SHR were significantly greater than that in Wistar rats. The inhibition magnitude of the contractile response by Ca^{2+}-activated Cl^- channel blocker, niflumic acid in SHR was significantly less than that in Wistar rats. Decreasing the extracellular Cl^- concentration increased the contractile response to NE significantly, but the amplitude of enhanced contractile response in SHR was greater than that in Wistar rats. It can be concluded that NE-induced contraction was enhanced in SHR, which is partly due to an increase in Cl^- efflux through the Ca^{2+}-activated Cl^- channels. The chloride channel activity may be increased in association with the elevation of blood pressure.

Key Words: calcium-activated chloride channels, norepinephrine, niflumic acid, spontaneous hypertensive rats

Introduction

The vascular mechanism of hypertension remains unclear. Essential hypertension is characterized by an increase in total peripheral resistance (TPR). TPR in turn is controlled directly by vessel diameter of the resistance arteries and arterioles. The change in vessel diameter is determined by the contractile state of vascular smooth muscle (VSM). In both human and animal models of essential hypertension, it has been demonstrated that the resting vascular tone is elevated, and the contractile response to a number of physiological stimuli is enhanced, one of which is the alteration of the permeability to ions of the membrane (14).

The relationship between blood pressure change and ion channel alteration has been addressed in many studies. Current experiment evidence indicates that K^+ channels, voltage gated Ca^{2+} channels (VDCC) and Cl^- channels (14, 20, 27, 29) are involved in the regulation of vascular tone and that the increased activity of Ca^{2+} channels and deceased activity of K^+ channels are responsible for the high contractile response of blood vessel in spontaneous hypertensive rats (SHR) (3, 10, 11, 22, 24, 26, 28). However, the change in activity and the role of Cl^- channels in hypertension is still unknown.

Recently numerous studies have attempted to define the role of Ca^{2+}-activated Cl^- channels ($Icl_{(Ca)}$) in the VSM. These studies have demonstrated that the intracellular Cl^- concentration, $[Cl^-]_i$, in VSM is in the range of 39-107 mmol/l, which is resulted from at

Corresponding author: Prof. Chen Lianbi, Institute of Physiology, School of Medicine, Shandong University, Jinan 250012, PR China. E-mail: clb@sdu.edu.cn. Tel: +86-531-8381910, Fax: +86-531-28382502
Received: November 19, 2003; Revised: April 9, 2004; Accepted: April 30, 2004.

least three mechanisms such as Na^+-K^+-$2Cl^-$ cotransport, Cl^--HCO_3^- exchange and ATP-dependent transport (2, 8, 9, 10). The equilibrium potential for Cl^- (E_{Cl}) is between -20 mV and -30 mV. Consequently, the opening of Cl^- channels causes Cl^- efflux, which enable the membrane to be polarized. The resulting membrane depolarization may in turn activate more VDCC to open, resulting in more Ca^{2+} inflow producing contraction. Therefore, research on the activity of the Ca^{2+}-activated Cl^- channels in VSM of SHR will be helpful for a good understanding of the episode of hypertension.

The present work was conducted to investigate the activity of Cl^- channel of the VSM in SHR to explore whether Cl^- channels do play a role in the development of hypertension, using *in vitro* perfused mesenteric microvasculature and tail artery strips techniques.

Materials and Methods

Animals

Twenty-five adult SHR and 25 Wistar rats both aged 7-8 weeks, male and female, and weighing 200 ± 50g were used. The SHR were provided by Chinese Academy of Medical Sciences, and age-matched Wistar rats were obtained from Shandong Academy of Medical Sciences. Systolic blood pressures in the conscious state were determined by an indirect tail-cuff technique. Before the experiments systolic blood pressure of SHR was 160±19 mmHg. During experiment the rats were anesthetized with pentobarbital sodium (40 mg/kg, ip).

Tissue Preparation

The mesenteric artery was carefully isolated from the intestines of the anesthetized rats. The isolated preparation of vascular bed was then suspended in water-jacketed chamber and perfused with physiological salt solution (PSS) maintained at 37 °C at constant flow (approximately 4-5 ml/min). The PSS was aerated with a mixture of 95% O_2-5% CO_2. Perfusion pressure was measured with a pressure transducer through a T tube placed between the pump and the mesenteric artery. Initial perfusion pressure was 25 mm Hg, and the preparation was allowed to equilibrate for 30 min before the start of experiments. At this level of perfusion pressure, the contractile response of the vascular bed to NE was examined in both rat groups. The reactivity of the mesenteric arteries was expressed as a percentage of the change in perfusion pressure.

The tail arteries were isolated from the anesthetized rats and then stored in cold PSS saturated with O_2 (4 °C). Under a dissecting microscope the arteries were cut helically into strips (2×10 mm). The helical strips were mounted vertically on a stainless steel holder in an organ chamber containing PSS aerated with a mixture of 95% O_2-5% CO_2. Before the start of experiments, the strips were allowed to equilibrate at 37 °C for 120 min in PSS. Isometric force transducer was connected to the strips and the isometric contraction was recorded. The contractile reactivity of the vessel strips to different chemicals was expressed as a percentage of 10^{-6} mol/l NE-induced maximum contraction on the same strips.

Protocols

To Induce Contraction with NE

Contractile responses of mesenteric vascular beds and tail arteries strips to NE were examined in all experiments, using the following two protocols. [1]. three or four different concentrations of NE were applied cumulatively to obtain a cumulative concentration-response curve, [2]. a single concentration of NE (10^{-6} mol/l) was added to elicit a transient maximum contraction.

To Observe Effect of Niflumic Acid on NE-Induced Contraction

Effect of niflumic acid on NE-induced contraction was investigated in the two types of arteries with the following patterns. 1. In mesenteric artery, the first three different concentrations of NE (10^{-6} mol/l, 5×10^{-6} mol/l, 10^{-5} mol/l) were added cumulatively to record the cumulative dose-response curve as a control recording. Once the curve was constructed, the tissue was washed with PSS every 20 min for 1 h and were then exposed to niflumic acid (10^{-4} mol/l) for 5 min. After that, the change in contractile responses to NE were recorded again. 2. In tail artery strips, the first four different concentrations of NE (10^{-9} mol/l, 10^{-8} mol/l, 10^{-7} mol/l and 10^{-6} mol/l) were added cumulatively to record the cumulative dose-response curve as a control recording. Then the effect of 10^{-4} mol/l niflumic acid on the above four concentrations of NE induced contraction response curve was observed. Finally, the preparation was exposed to four different concentrations of niflumic acid (10^{-6} mol/l, 10^{-5} mol/l, 5×10^{-5} mol/l, 10^{-4} mol/l) for 10 min and then record the change in contractile response to 10^{-6} mol/l NE.

To Observe Effect of Decreasing $[Cl^-]_o$ on NE-Induced Contraction

Low Cl^- buffer (8 mmol/l) was prepared by the replacement of 130 mmol/l NaCl with 130 mmol/l

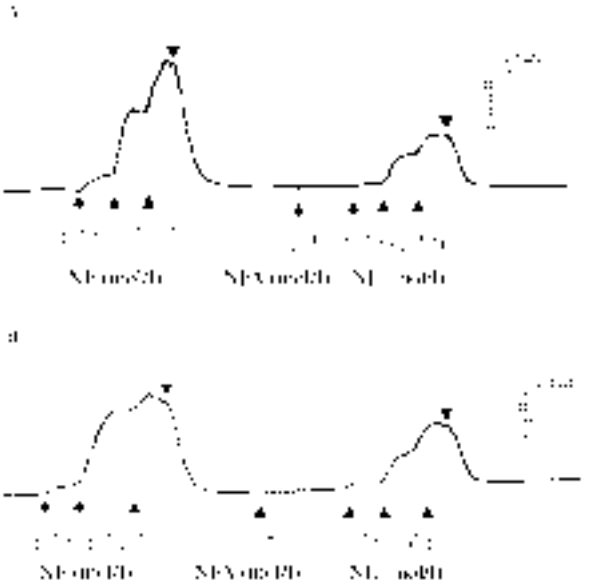

Fig. 1. Effect of 10^{-4} mol/l niflumic acid on NE-induced contraction of mesenteric vascular beds in Wistar rats (A) and SHR (B). Typical trace showing the inhibitory effect of 10^{-4} mol/l niflumic acid (NFA) on the contraction of mesenteric vascular beds induced by the cumulative application of NE (▼ wash).

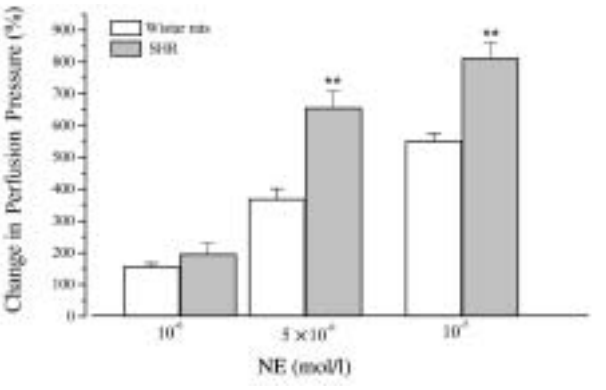

Fig. 2. NE-induced contraction of mesenteric vascular beds in Wistar rats and SHR. The contractile responses to NE were greater in the preparations from SHR than in those from Wistar rats. **: $P < 0.01$ *vs.* Wistar rats, Values are expressed $\bar{x} \pm s$, $n = 10$.

NaOH. The pH of the buffer was titrated to 7.4 with methanesulfonic acid. Contractile response to NE in the low concentration Cl^- buffer was recorded to compare with those obtained in response to NE in normal Cl^- buffer, PSS (138 mmol/l Cl^-).

Drugs

NE was purchased from Shanghai Hefeng Pharmaceutical Company LTD. Stock solution was 10^{-3} mol/l in distilled water.

Niflumic acid, a Ca^{2+}-activated Cl^- channel blocker (4, 5), was purchased from Sigma. Stock solution was 1 mol/l in DMSO.

Data Analysis

The data was expressed as mean±SE. Statistical analysis of group differences was performed using *t*-test. A value of $P < 0.05$ was considered to be statistically significant.

Results

NE-Induced Contraction of Mesenteric Vascular Bed

Infusion of NE (10^{-6} mol/l, 5×10^{-6} mol/l, 10^{-5} mol/l) produced a dose-dependent contraction in mesenteric preparation from SHR and Wistar rats (Fig. 1, 2). The contractile responses to NE were greater in the preparations from SHR than in those from Wistar rats (see Fig. 2). However, statistically greater responses were observed in SHR at the NE concentration of 5×10^{-6} mol/l and 10^{-5} mol/l. These results suggested that the contractile reactivity of mesenteric vascular beds to NE was increased in SHR.

Effect of 10^{-4} M Niflumic Acid on NE-Induced Contraction of Mesenteric Vascular Beds

Niflumic acid (10^{-4} mol/l) reduced the NE-induced contraction markedly at the three different concentrations of NE in SHR and Wistar rats (Fig. 1), which suggested that niflumic acid blocked partly the contraction produced by NE and the niflumic acid sensitive Cl^- channels ($Icl_{(Ca)}$) contribute to NE-induced contraction.

It was also observed that the inhibitory magnitude of the contractile response by niflumic acid in SHR was significantly less than that in Wistar rats at the three concentrations of NE (Fig. 3). These results indicated that relaxation response of mesenteric arteries in SHR was less than that of Wistar rats ($P < 0.05$) and indicated that the activity of niflumic acid sensitive Cl^- channels in VSM of SHR was more active than in normotensive control rats.

Effect of Decreasing $[Cl^-]_o$ on NE-Induced Contraction of Mesenteric Vascular Beds

Fig. 4 is an original trace of NE-induced contraction in low and normal Cl^- buffer solutions, which shows that the contractile response to 10^{-6} mol/l NE was enhanced in low Cl^- buffer (8 mmol/l) markedly both in SHR and Wistar rats, but the amplitude of enhanced contraction response in SHR was greater

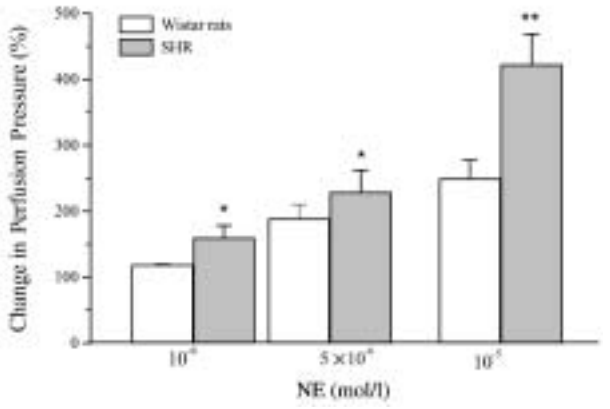

Fig. 3. Effect of 10^{-4} mol/l niflumic acid on NE-induced contraction of mesenteric vascular beds in Wistar rats and SHR. *: $P < 0.05$, **: $P < 0.01$ *vs.* Wistar rats , values are expressed as $\bar{x} \pm s$, $n = 10$.

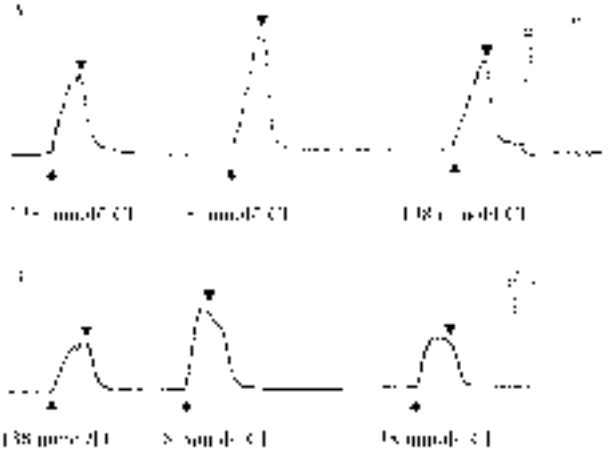

Fig. 4. Effect of low Cl^- buffer on 5×10^{-6} mol/l NE-induced contraction of mesenteric vascular beds in Wistar rats (A) and SHR (B). Typical response of a vascular bed to reduced extracellular Cl^- concentration. Low-Cl^- buffer (8 mmol/l) significantly enhanced the NE-induced contraction and the effect was reversible when the buffer was changed back to the normal Cl^- solution (▼ wash).

than that in Wistar rats (Fig. 4, 5).

Effect of 10^{-4} M Niflumic Acid on NE-Induced Contraction of Tail Arteries

Adding four different concentrations of NE (10^{-9} mol/l, 10^{-8} mol/l, 10^{-7} mol/l and 10^{-6} mol/l) produced dose-dependent contraction of the tail arteries in SHR and Wistar rats (Fig. 6). Niflumic acid (10^{-4} mol/l) decreased the maximum contraction by 48% in Wistar rats and 41% in SHR, respectively (Fig.7). The inhibition magnitude of the contractile response by niflumic acid in SHR was significantly less than that in Wistar rats at two NE concentrations of 10^{-7} mol/l and 10^{-6} mol/l (Fig. 7).

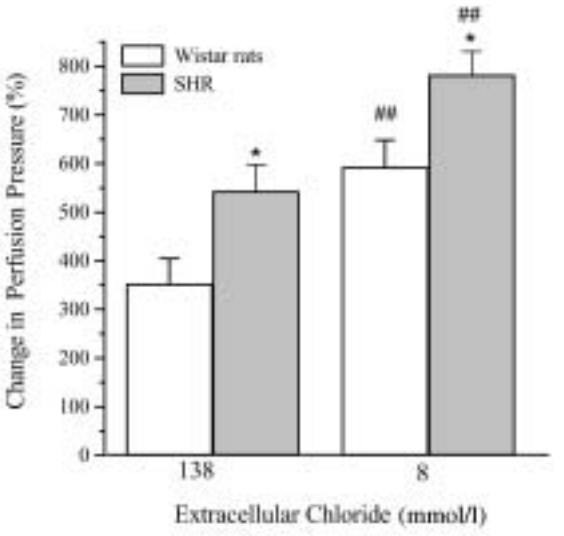

Fig. 5. Effect of low Cl^- buffer on 5×10^{-6} mol/l NE-induced contraction of mesenteric vascular beds in Wistar rats and SHR. The amplitude of enhanced contraction response in SHR was greater than that in Wistar rats. *: $P < 0.05$ *vs*. Wistar rats, # # : $P < 0.01$ *vs.* PSS, values are expressed as $\bar{x} \pm s$, $n = 10$.

Effect of Different Concentrations of Niflumic Acid on 10^{-6} M NE-Induced Contraction of Tail Arteries

Fig. 8 shows a typical recording of a rapid development of tension in the strips of tail arteries produced by 10^{-6} mol/l NE. Niflumic acid produced a dose-dependent inhibitory effect on the NE-induced contraction. 10^{-4} mol/l niflumic acid inhibited NE-induced transient contractions by 53% in Wistar rats and 42% in SHR, respectively (Fig. 9). The inhibition magnitude of the contractile response by niflumic acid in SHR was significantly less than that in Wistar rats (Fig. 9).

Discussion

The Evidences of Enhancement of Activity of the Ca^{2+}-Activated Cl^- Channels in SHR

The data in the present study support the hypothesis that the activity of $Icl_{(Ca)}$ is altered in mesenteric vascular beds and tail arteries in SHR and that this may play a role in the initiation of high blood pressure. Three pieces of evidence established by this study are as follows. First, the contractile responses of mesenteric arteries and tail arteries to NE in SHR were greater than that in Wistar rats. Second, the inhibition magnitude of the contractile response by Ca^{2+}-activated Cl^- channel blocker, niflumic acid in SHR was significantly less than that in Wistar rats. Third, lower extracellular Cl^- concentration increased

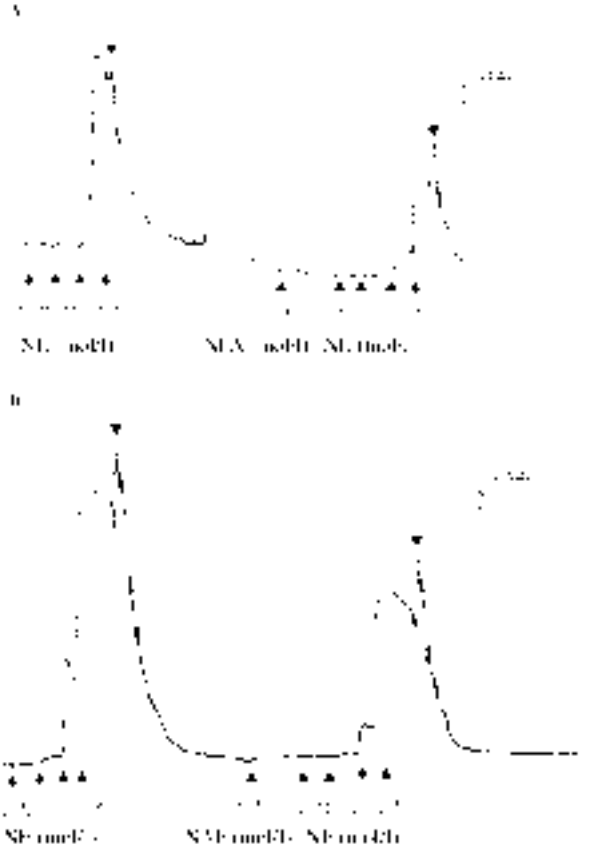

Fig. 6. Effect of 10^{-4} mol/l niflumic acid (NFA) on NE-induced contraction of tail arteries in Wistar rats (A) and SHR (B). Typical trace showing the inhibitory effect of 10^{-4} mol/l niflumic acid (NFA) on the contraction of tail arteries induced by the cumulative application of NE (▼ wash). *: $P < 0.05$ *vs.* Wistar rats, values are expressed as $\bar{x} \pm s$, $n = 10$.

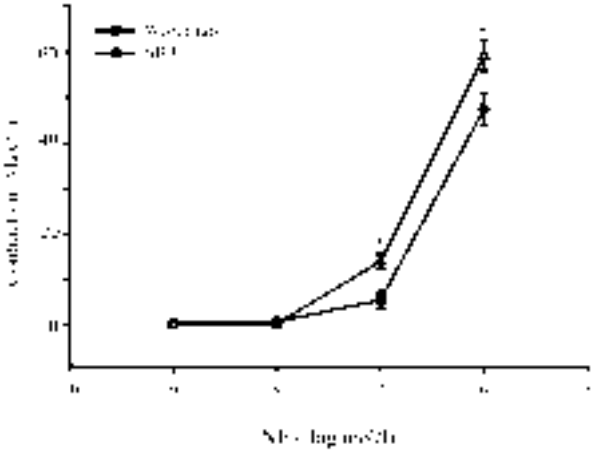

Fig. 7. Effect of 10^{-4} mol/L niflumic acid on NE-induced contraction of tail arteries in Wistar rats and SHR. *, $P < 0.05$ *vs.* Wistar rats, values are expressed as $\bar{x} \pm s$, $n = 10$.

the contractile response to NE significantly and the amplitude of enhanced contractile response in SHR was greater than that in Wistar rats.

Ca^{2+}-Activated Cl^- Channels Contribute NE-Induced Contraction

In the recent years, niflumic acid have been shown to activate the large Ca^{2+}-activated K^+ (BKca) channels (6, 25). Activity of BKca channels were also shown to play a central role in the regulation of vascular tone due to focal increase in subsarcolemmal Ca^{2+} (i.e., Ca^{2+} sparks) by Ca^{2+} released through ryanodine receptors in the sarcoplasmic reticulum of the VSM (16, 17, 23). The BKca channels are activated during active vasoconstriction by agents such as NE (15). However, Criddle have investigated the effects of niflumic acid in the isolated aorta (4) and mesenteric vascular bed of normal rats (5). His research suggested that niflumic acid does not directly block Ca^{2+} channels or activate K^+ channels.

The expression of BKca channels in VSM membrane is increased during hypertension as a negative feedback response to the increased vascular reactivity (1, 21). However, data from the present work showed that the inhibition of the NE induced contraction of the mesenteric and tail arteries by 10^{-4} mol/l niflumic acid in Wistar rats are even greater than that in SHR, which corresponds with the experiment results reported by Criddle and Hong *et al*. (4, 5, 12, 13, 18). In addition, after adding niflumic acid the basic vascular tone both of mesenteric vascular beds and tail artery strips were not altered at all. The effect of niflumic acid on VSM is dose-dependent in the present experiment. When lower chloride concentration in buffer opened up more chloride channels, the contractile response of VSM to NE increased more in SHR than that in normal rats, indicating an enhanced activity of the chloride channels of VSM in SHR. BKca channels have been shown to have a high Ca^{2+} threshold in relaxed VSM, with a higher level of Ca^{2+} 3 to 10 mol/l (15). Therefore, the above evidence suggests that in the present experiment, the inhibitory effects of external niflumic acid on VSM constriction is not due to activation of the BKca channels and that Ca^{2+}-activated chloride channels may play a pivotal role in the activation of VDCC in NE-induced contraction of the resistance blood vessels.

Mechanism of the Enhanced NE-Induced Contraction by Low $[Cl^-]_o$

It is well known that both ionic permeabilities and concentration gradients determine the transmembrane potential and excitation of VSM. The methanesulfonic ion is an impermeant anion and can be used as the impermeable Cl^- substitute to alter the Cl^-

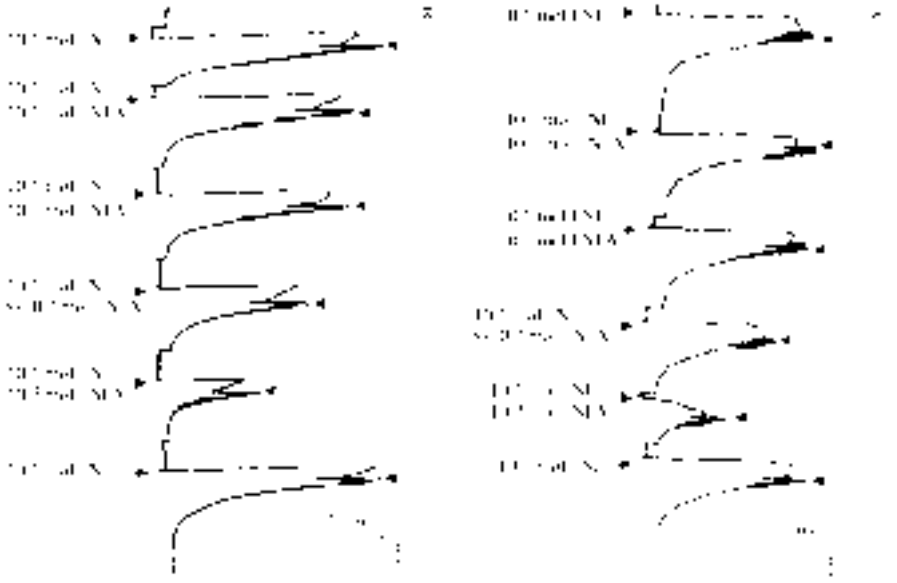

Fig. 8. Effect of different concentrations of niflumic acid (NFA) on 10^{-6} mol/l NE-induced contraction of tail arteries in Wistar rats (A) and SHR(B). Typical trace showing the inhibitory effect of 10^{-6} , 10^{-5}, 5×10^{-5}, 10^{-4} mol/l niflumic acid (NFA) on the contraction of tail arteries induced by 10^{-6} mol/l NE (▼ wash). *: $P < 0.05$ *vs.* Wistar rats, values are expressed as $\bar{x} \pm s$, $n = 10$.

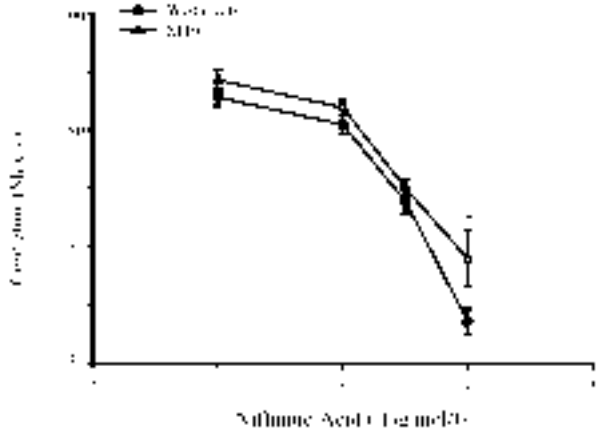

Fig. 9. Effect of the different concentration niflumic acid on 10^{-6} mol/l NE-induced contraction of tail arteries in Wistar rats and SHR. *: $P < 0.05$ *vs.* Wistar rats, values are expressed as $\bar{x} \pm s$, $n = 10$.

concentration (7, 19, 30). We found that low Cl^- buffer solution significantly enhanced the NE-induced vasocontraction. This is resulted from the increased Ca^{2+} entry because lower $[Cl^-]_o$ might increase Cl^- efflux and thus the membrane depolarization would open up more VDCC. Dives (10) reported a significant rise in intracellular Cl^- in VSM of femoral artery in the deoxycorticosterone acetate (DOCA-salt) hypertension rats, which was due to an increase of the activity of Na^+-K^+-$2Cl^-$ cotransport and might facilitate the VSM.

In conclusion, our data suggested that activity of $Icl_{(Ca)}$ of VSM in SHR was enhanced, which increased vascular reactivity to NE. This might be one of the factors to induce and maintain the higher vascular tone and peripheral resistance in hypertension.

Acknowledgments

This study was greatly supported by Prof. Xiangshu Chai, Department of Physiology, Shandong Academy of Medical Sciences, Shandong, and supported by the grant of public health from Department of Public Health of Shandong Province.

References

1. Asano, M., Matsuda, T., Hayakawa, M., Ito, K.M. and Ito, K. Increased resting calcium maintains the myogenic tone and activates K^+ channels in arteries from young spontaneously hypertensive rats. *Eur. J. Pharmacol*. 247: 295-304, 1993.
2. Chipperfiled, A.R., Davis, J.P. and Harper, A.A. An acetazolamide-sensitive inward chloride pump in vascular smooth muscle. *Biochem. Biophys. Res. Commum*. 194: 407-412, 1993.
3. Cox, R.H. and Lozinskaya, I.M. Augmented calcium currents in mesenteric artery branches of the spontaneously hypertensive rat. *Hypertension* 26: 1060-1064, 1995.
4. Criddle, D.N., de Moura, R. S., Greenwood, I.A. and Large, W.A. Effect of niflumic acid on noradrenaline-induced contractions of the rat arota. *Br. J. Pharmacol*. 118: 1065-1071, 1996.
5. Criddle, D.N., de Moura, R. S., Greenwood, I. A. and Large, W.A. Inhibitory action of niflumic acid on noradrenaline- and 5-hydroxytryptamine-induced pressor responses in the isolated mesenteric vascular bed of the rat. *Br. J. Pharmacol*. 120: 813-818, 1997.

6. Cruickshank, S.F., Baxter, L.M. and Drummond, R.M. The Cl^- channel blocker niflumic acid releases Ca^{2+} from an intracellular store in rat pulmonary artery smooth muscle cells. *Br. J. Pharmacol.* 140: 1442-1450, 2003.
7. Dai, Y. and Zhang, J.H. Role of Cl^- current in endothelin-1-induced contraction in rabbit basilar artery. *Am. J. Physiol. (Heart Cirl. Physiol.)* 281: H2159-H2167, 2001.
8. Divis, J.P. The effects of Na^+-K^+-Cl^- co-transport and Cl^--HCO_3^- exchange blockade on the membrane potential and intracellular chloride levels of rat arterial smooth muscle, in vitro. *Exp. Physiol.* 77: 857-862, 1992.
9. Davis, J.P.L. Evidence against a contribution by Na-Cl cotransport to chloride accumulation in rat arterial smooth muscle. *J. Physiol. (Lond.)* 491: 61-68, 1996.
10. Davis, J.P., Chipperfield, A.R. and Harper, A.A. Accumulation of intracellular chloride by (Na-K-Cl) co-transport in rat arterial smooth muscle is enhanced in deoxycorticosterone acetate (DOCA)/ salt hypertension. *J. Mol. Cell. Cardiol*. 25: 233-237, 1993.
11. England, S.K., Wooldridge, T.A., Stekiel, W.J. and Rusch, N.J. Enhanced single-channel K^+ current in arterial membranes from genetically hypertensive rats. *Am. J. Physiol*. 264: H1337-1345, 1993.
12. Hogg, R.C., Wang, Q. and Large, W.A. Action of niflumic acid on evoked and spontaneous calcium-activated chloride and potassium currents in smooth muscle cells from rabbit portal vein. *Br. J. Pharmacol*. 112: 997-984, 1994.
13. Hogg, R.C., Wang, Q. and Large, W.A. Effects of Cl channel blockers on Ca-activated chloride and potassium currents in smooth muscle cells from rabbit portal vein. *Br. J. Pharmaco*l. 111: 1333-1341, 1994.
14. Jackson, W.F. Ion channels and vascular tone. *Hypertension* 35: 173-178, 2000.
15. Jackson, W.F. and Blair, K.L. Characteristics and function of Ca^{2+}-activated K^+ channels in hamster cremasteric arteriolar muscle cells. *Am. J. Physiol. (Heart Cirl. Physiol.*) 274: H27-H34, 1998.
16. Jaggar, J.H., Wellman, G.C., Heppner, T.J., Porter, V.A., Perez, G.J., Gollasch, M., Kleppisch, T., Rubart, M., Stevenson, A.S., Lederer, W.J., Knot, H.J., Bonev, A.D. and Nelson, M.T. Ca^{2+} channels, ryanodine receptors and Ca^{2+}-activated K^+ channels: a functional unit for regulating arterial tone. *Acta. Physiol. Scand.* 164: 577-587, 1998.
17. Knot, H.J., Standen, N.B. and Nelson, M.T. Ryanodine receptors regulate arterial diameter and wall [Ca^{2+}] in cerebral arteries of rat Ca^{2+}-dependent K^+ channels. *J. Physiol. (Lond.)* 508: 211-221, 1998.
18. Lamb, F.S. and Barna, T.J. Chloride ion currents contribute functionally to norepinephrine-induced vascular contraction. *Am. J. Physiol. (Cell. Heart Circ. Physiol*.) 275: H151-H160, 1998.
19. Lamb, F.S., Volk, K.A. and Shibata, E.F. Calcuim-activated chloride current in rabbit coronary artery myocytes. *Circ. Res.* 75: 742-750, 1994.
20. Large, W.A. and Wang, Q. Characteristics and physiological role of the Ca^{2+}-activated Cl^- conductance in smooth muscle. *Am. J. Physiol. (Cell. Physiol*.) 271: C435-454, 1996.
21. Liu, Y., Pleyte, K., Knaus, H.G. and Rusch, N.J. Increased expression of Ca^{2+}-sensitive K^+ channels in aorta of hypertensive rats. *Hypertension* 30: 1403-1409, 1997.
22. Lozinskaya, I.M. and Cox, R.H. Effects of age on Ca^{2+} currents in small mesentericartery myocytes from Wistar-Kyoto and spontaneously hypertensive rats. *Hypertension* 29: 1329-1336, 1997.
23. Nelson, M.T., Cheng, H., Rubart, M., Santana, L.F., Boney, A.D., Knot, H.J. and Ledere, W.J. Relaxation of arterial smooth muscle by calcium sparks. *Science* 270: 633-637, 1995.
24. Ohya, Y., Abe, I., Fujii, K., Takata, Y. and Fujishima, M. Voltage-dependent Ca^{2+} channels in resistance arteries from spontaneously hypertensive rats. *Circ. Res*. 73: 1090-1099, 1993.
25. Ottolia, M. and Tolo, L. Potentiation of large conductance KCa channels by niflumic acid, flufenamic, and mefenamic acid. *Biophys. J.* 67: 2272-2279, 1994.
26. Rusch, N.J. and Hermsmeyer, K. Calcium currents are altered in the vascular muscle cell membrane of spontaneously hypertensive rats. *Circ. Res*. 63: 997-1002, 1988.
27. Suyin, A., Min, L., Stapleton, M.P. and Tabrizchi, R. Influence of chloride ions on α-adrenoceptor mediated contraction and Ca^{2+} influx in rat caudal artery. *Life Sci*. 64: 1631-1641, 1999.
28. Wilde, D.W., Furspan, P.B. and Szocik, J.F. Calcium current in smooth muscle cells from normotensive and genetically hypertensive rats. *Hypertension* 24: 739-746, 1994.
29. Yamazali, J., Duan, D., Janiak, R., Kuenzli, K., Horowitz, B. and Hume, J.R. Functional and molecular expression of volume-regulated chloride channels in canine vascular smooth muscle cells. *J. Physiol. (Lond.)* 507: 729-736, 1998.
30. Zygmunt, A.C. and Gibbons, W.R. Calcium-activated chloride current in rabbit ventricular myocytes. *Circ. Res*. 68: 424-437, 1991.