J. Ginseng Res.
Vol. 26, No. 1, 1-5(2002)

Vasorelaxing Mechanism of Crude Saponin of Korea Red Ginseng in the Resistance-sized Mesenteric Artery of Rat

Shin Hye Kim, Hyung Seo Park, Mee Young Lee, Young Sun Oh* and Se Hoon Kim#

Department of Physiology, College of Medicine, Konyang University, Nonsan, Chungnam, 320-711, Korea
**College of Oriental Medicine, Daejeon University, Daejeon, 301-131, Korea*
(Received October 11, 2001)

Abstract : It has been well known that Korea red ginseng has an antihypertensive effect. The antihypertensive effect may be due to its ability to change the peripheral resistance. Change of vascular tone in the resistance-sized artery contribute to the peripheral resistance, thereby regulate the blood pressure. Therefore, we investigated to clarify the vasorelaxing mechanism induced by crude saponin of Korea red ginseng in the resistance-sized mesenteric artery of rats. The resistance-sized mesenteric artery was isolated and cut into a ring. The ring segment was immersed in HEPES-buffered solution and its isometric tension was measured using myograph force-displacement transducer. Crude saponin of ginseng relaxed the mesenteric arterial rings precontracted with norepinephrine (3 μM) in dose-dependent manner (0.01 mg/m*l* ~ 1 mg/m*l*). The relaxation by crude saponin was smaller in endothelium-intact preparation than that in endothelium-denuded preparation. The contraction induced by A23187 or phorbol 12,13-dibutyrate was not affected by crude saponin of ginseng. The vasorelaxing effect of crude saponin of ginseng was significantly attenuated by the increase of the extracellular K^+ concentration. Crude saponin-induced vasorelaxation was not affected by tetraethylammonium (1 mM), glybenclamide (10 μM), and 4-aminopyridine (0.1 mM) in these preparations. Ba^{2+} (10 μM ~ 100 μM) markedly reduced the crude saponin-induced vasorelaxation dose-dependently. From the above results, we suggest that crude saponin of ginseng may stimulate K^+ efflux and hyperpolarize the membrane, thereby cause the vasorelaxation in the resistance-sized mesenteric artery of rats.

Key words: Vasorelaxation, antihypertensive effect, peripheral resistance, resistance-sized mesenteric artery

INTRODUCTION

Korea red ginseng has long been used in Korean traditional medicine. It has been well known that Korea red ginseng has an antihypertensive effect.[1,2] The blood pressure-lowering effect of ginseng may be due, at least in part, to its vasorelaxing effect in the several kinds of vessels. There are several reports about the vasorelaxing effects of ginseng. Ginsenosides relax isolated rabbit pulmonary artery and canine mesenteric vein.[3] Ginsenoside Rg3 relax the rat aorta in endothelium-dependent and -independent manner.[4-6]

Small artery contributes to the majority of resistance to blood flow. Therefore vasomotor tone of small artery is a major factor in blood pressure regulation. However, most studies of ginseng in vascular smooth muscle have been conducted on isolated conduit vessels and studies of ginseng in resistance-sized small arteries have not been reported. It has been suggested that vasorelaxation by ginseng is mediated via the activation of K^+ channel. Ca^{2+}-activated K^+ channel seems to be implicated in ginseng-induced vasorelaxation of conduit vessel.[6,7] The distribution of K^+ channel varies according to the size of the vessel and the organ in which the vessels are located.[8] Therefore it is necessary to clarify the underlying mechanisms of vasorelaxation induced by ginseng in the resistance-sized artery.

The objective of present study was to determine the mechanism by which ginseng relax the resistance-sized mesenteric artery of the rat. Specifically, we were interested in what types of K^+ channel is involved in vasorelaxation by crude saponin of ginseng.

MATERIALS AND METHODS

Twenty-week old Wistar Kyoto rats (250-300 g) were anesthetized by ether and killed by bleeding from the common carotid artery. The second-branch of superior

To whom correspondence should be addressed.
(Tel) 041-730-5344; (Fax) 041-736-5318
(E-mail) sehkim@konyang.ac.kr

mesenteric artery was isolated and the surrounding connective tissue was removed. The diameter of mesenteric artery was less than 150 μm. Arterial rings about 2 mm in length were prepared and mounted between two stainless steel hooks in 5 ml organ bath containing HEPES-buffered solution of the following composition (mM) : NaCl, 143; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.0; glucose, 5; HEPES, 10. The HEPES-buffered solution was titrated with NaOH to pH 7.4. In experiments using high-K^+ solution, Na^+ in the bathing medium was replaced by equimolar concentration of K^+ to maintain a constant ion strength. The bath solution was constantly bubbled with 100% O_2. The isometric tension was measured with a myograph force-displacement transducer (Myo-interface, Model 410A, Denmark) and recorded with a strip chart recorder (Linear).

The arterial ring was allowed to equilibrate for 60 minutes under 3 mN resting tension. The endothelium was removed by rubbing the inner surface using hair. Removal of endothelium was confirmed when the mesenteric artery did not show any vasorelaxation response to acetylcholine. The arterial ring was contracted with single concentration of norepinephrine (NE, 3 μM) three times before experiment. After we confirmed that the amplitude of NE-induced contraction was constant, experiments were performed. The arterial ring was incubated with each inhibitor for 15 minutes before they were contracted with agonists, then crude saponin of ginseng was added. The vasorelaxation effects of crude saponin of ginseng on the sustained tone were expressed as percentages of the maximal contraction induced by NE.

The following compounds were used: crude saponin of ginseng, norepinephrine hydrochloride, A23187, phorbol 12,13-dibutyrate, tetraethylammonium, 4-aminopyridine, glybenclamide and $BaCl_2$. All compounds except A23187 and glybenclamide were dissolved in distilled water. A23187 was dissolved in ethanol and glybenclamide was dissolved in DMSO. A23187 and glybenclamide were diluted in HEPES-buffered solution before use.

RESULTS

NE (3 μM) induced a sustained contraction and crude saponin of ginseng induced a concentration-dependent relaxation in endothelium-denuded or endothelium-intact mesenteric artery as shown in Fig. 1. The relaxation by crude saponin was smaller in endothelium-intact preparation than that in endothelium-denuded preparation. However, the contraction induced by A23187 or phorbol 12,13-dibutyrate (PDBu) was not affected by crude saponin of ginseng (Fig. 2).

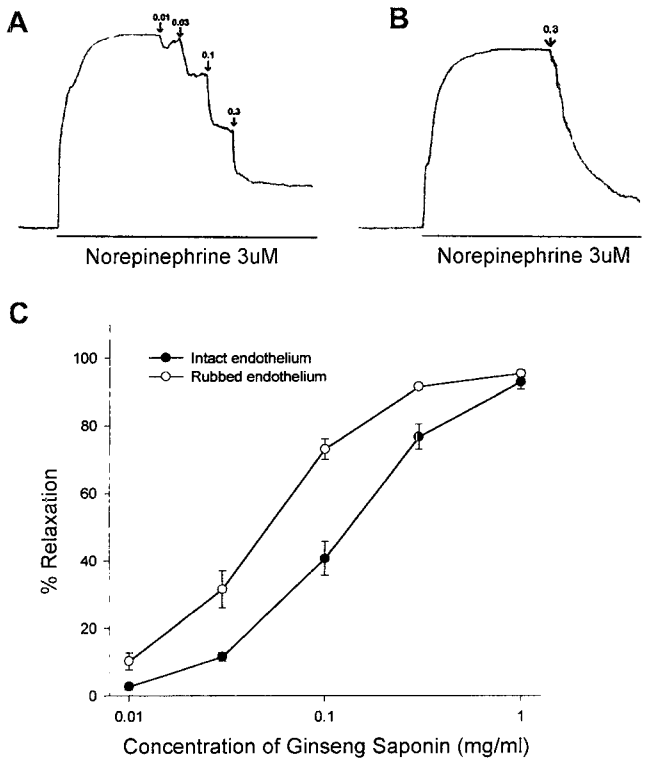

Fig. 1. The relaxant effect of crude saponin of ginseng in the mesenteric artery of rat. The trace shows a representative record of the relaxant effect of crude saponin in an artery with endothelium (A) and rubbed endothelium (B). C; Dose-dependent relaxation by crude saponin in the norepinephrine-precontracted mesenteric artery. Data are means ± S.E. of 6 experiments.

In high potassium medium (KCl=25 mM or 45 mM), we observed crude saponin-induced vasorelaxation of NE-precontracted preparations without endothelium. Fig. 3 shows that the crude saponin-induced vasorelaxation was greatly reduced in high potassium medium. In 5 mM K^+ medium, the relaxation by crude saponin (0.6 mg/ml) was 90.6± 2.03%, and the relaxations were attenuated to 66.3 ± 2.88% and 31.5± 4.69% in 25 mM and 45 mM K^+ medium, respectively.

The relaxant effect of crude saponin on induced tone was examined in endothelium-denuded preparations pretreated with various K^+ channels blockers, tetraethylammonium, 4-aminopyridine, glybenclamide and Ba^{2+}. Pretreatment with tetraethylammonium (1 mM), blocker of Ca^{2+}-activated K^+-channel, did not affect crude saponin-induced relaxation in the NE-precontracted endothelium-denuded preparations. 4-Aminopyridine (1 mM), blocker of voltage-dependent K^+-channel, also did not alter the

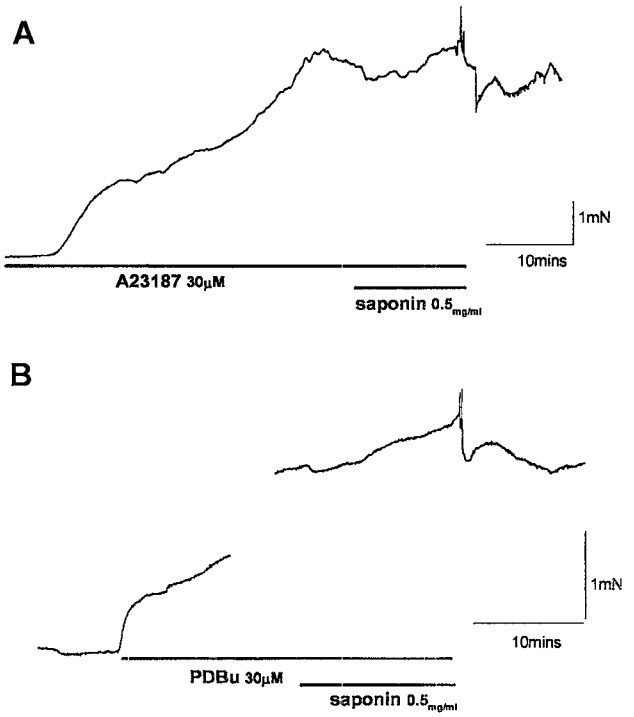

Fig. 2. The relaxant effect of crude saponin of ginseng on the A23187 (A) or PDBu (B)-precontracted mesenteric artery without endothelium.

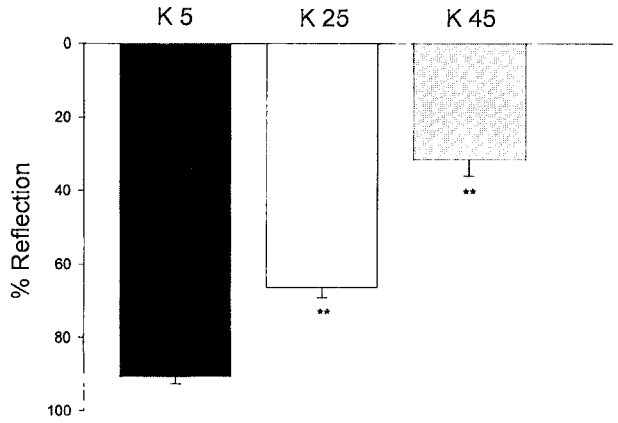

Fig. 3. Effects of extracellular K^+ concentration on the relaxant effect of crude saponin in the norepinephrine-precontracted mesenteric artery without endothelium. Data are means ± S.E. of 6 experiments. **P<0.01: Significantly different from K5.

crude saponin-induced relaxation. Glybenclamide, blocker of ATP-sensitive K^+ channel, at 10 μM did not alter the crude saponin-induced relaxation (Fig. 4). In contrast, Ba^{2+} significantly reduced the crude saponin-induced vasorelaxation dose-dependently. The relaxations by crude saponin (0.6 mg/ml) were reduced to 81.6± 4.18%, 39.1

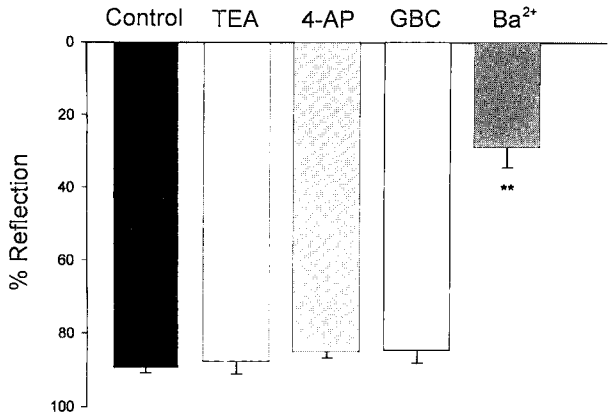

Fig. 4. Effects of K^+ channel blockers on the relaxant effect of crude saponin in the norepinephrine-precontracted mesenteric artery without endothelium. Data are means ± S.E. of 6 experiments. ***P<0.01: Signiticantly different from control.

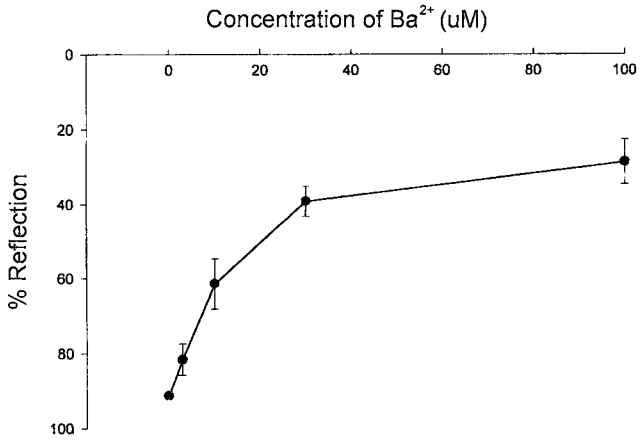

Fig. 5. Effects of Ba^{2+} on the relaxant effect of crude saponin in the norepinephrine-precontracted mesenteric artery without endothelium. Data are means ± S.E. of 6 experiments.

± 4.03% and 28.6± 5.93% for 3 μM, 30 μM and 100 μM Ba^{2+} from 91.3± 0.69%, respectively (Fig. 5).

DISCUSSION

Several studies reported that ginsenosides causes endothelium-dependent relaxation of vascular smooth muscle and is also able to inhibit vascular smooth muscle contraction directly.[5,6,9] The present study also demonstrates that crude saponin of ginseng is able to evoke a endothelium-independent relaxation in the isolated resistance-sized mesenteric artery of a rat. Vasorelaxation is induced by 1) reduced sensitivity of contractile protein to Ca^{2+}, 2) hyperpolarization of membrane potential, 3) the direct inhibition of Ca^{2+} channels. PDBu-dependent activation

of PKC in arterial preparations increases the sensitivity of the contractile apparatus to Ca^{2+}, thereby cause vasoconstriction without increasing intracellular $[Ca^{2+}]$.[10] NE is known to not only increase the influx of Ca^{2+} via the activation of voltage-gated Ca^{2+} channel but also increase the sensitivity of the contractile apparatus to Ca^{2+}, thereby potentiate the contraction.[10] A23187, Ca^{2+} ionophore, makes Ca^{2+}-selective pores and increases Ca^{2+} influx, thereby causing vasoconstriction. Crude saponin inhibited the contraction induced by NE but it did not affect the PDBu- or A23187-induced contraction. Present findings indicate that crude saponin did not exert any effect on the Ca^{2+} sensitivity of contractile proteins and the Ca^{2+} influx via Ca^{2+} pores in resistance-sized mesenteric artery. Previous studies with rat sensory neurons[11] and cultured rat ventricular myocytes[12] have suggested that ginsenosides have direct Ca^{2+}-channel blocking effects. And Kim *et al.*[6] suggested the possibility that inhibition of voltage-dependent Ca^{2+} channels is one of the causes of vasorelaxant effects of ginsenosides. In the present study, crude saponin inhibited the NE-induced contraction dose-dependently but its relaxation effect is reduced in high K^+ medium. These findings minimize the possibility that crude saponin might inhibit the Ca^{2+} channel directly. Instead, crude saponin may activate K^+ efflux and cause the membrane hyperpolarization, thereby inhibit Ca^{2+} influx through voltage-gated Ca^{2+} channels. There is no evidence that Na^+ channels are present in vascular smooth muscles. Thus, membrane potential of vascular smooth muscle is determined by K^+ conductance and the K^+ concentration gradient. The increase of K^+ conductance hyperpolarize the membrane potential and the decrease of K^+ concentration gradient hypopolarize the membrane potential. At 25~40 mM of extracellular $[K^+]$, the equilibrium potential for K^+ would be more negative than the membrane potential depolarized by NE. Under this condition, increase of K^+ conductance will hyperpolarize the membrane potential because the ensuing hyperpolarization due to K^+ conductance increase overcomes the effect of hypopolarization by the decrease in the K^+ concentration gradient. Therefore, at 25~40 mM of extracellular $[K^+]$, crude saponin-induced vasorelaxation was partially attenuated. In the contrary, at 60 mM of extracellular $[K^+]$, increase of K^+ conductance could not hyperpolarize the membrane potential and crude saponin did not relax these preparations. Therefore it is suggested that the opening of K^+ channels in vascular smooth muscle and subsequent hyperpolarization of that cell mediate mesenteric arterial dilation in response to crude saponin.

A variety of K channels exist in smooth muscle cells of arteries, including Ca^{2+}-activated K^+ channel,[13] voltage-dependent or delayed rectifier K^+ channel,[14] ATP-sensitive K^+ channel[15] and the inward rectifier K^+ channel.[16] Their distribution varies according to the size of the vessel and the organ in which the vessels are located. Among them, ATP-sensitive K^+ channel,[15] Ca^{2+}-activated K^+ channel[17] and voltage-dependent K^+ channel[18,19] have been identified in resistance-sized rat mesenteric artery. Li[7] and Kim[5,6] reported that Ca^{2+}-activated K^+ channel is a major target of ginsenoside in vasorelaxation of conduit artery such as aorta and large artery. However, in the present study, crude saponin-induced relaxation was inhibited by Ba^{2+} but vasorelaxation by crude saponin was not influenced by tetraethylammonium, 4-aminopyridine and glybenclamide in resistance-sized rat mesenteric artery. This results indicate that the activation of ATP-sensitive K^+ channel, Ca^{2+}-activated K^+ channel and voltage-dependent K^+ channel may not be involved in the mediating the relaxation by crude saponin. Ba^{2+} is an effective blocker of inward rectifier K-channel at micromolar concentration.[20,21] In the present study, crude saponin-induced vasorelaxation was inhibited at micromolar concentration (10 ~ 100 μM) of Ba^{2+}. However, there is no evidence that inward rectifier K^+ channel is present in resistance-sized mesenteric artery. In some reports, Ba^{2+} inhibit K^+ channel nonspecifically. We observed that Ba^{2+} inhibit SNAP- or isoproterenol-induced vasorelaxation in resistance-sized mesenteric artery of rat (data not shown). SNAP is known to cause vasorelaxation via activation of Ca^{2+}-activated K^+ channel by cGMP formation and isoproterenol stimulate cAMP formation and activate ATP-sensitive K^+ channel. Therefore, it remains to be determined whether the inward rectifier K^+ channel is present or Ba^{2+} inhibit K^+ channel nonspecifically in the resistance-sized mesenteric artery.

Therefore, it is suggested that the suppression of contractility of rat resistance-sized mesenteric artery caused by crude saponin appears to be due to the K^+ channel opening effect of this substance. Crude saponin activate K^+ channel which is sensitive to Ba^{2+} and hyperpolarize the membrane potential, which result in inhibition of voltage-gated Ca^{2+} channel. The type of K^+ channel involved in crude saponin-induced vasorelaxation is not certain. Further studies are needed to elucidate the identity of the specific K^+ channels involved and further investigation is needed to explain the underlying mechanisms for regulation of K^+ channel by crude saponin.

요 약

고려홍삼은 혈압강하효과가 있음이 잘 알려져 있다. 이에 백서 장간막 동맥의 저항혈관에서 고려홍삼 사포닌 성분의 혈관 이완 기전을 규명하고자 내경이 150 μm이하의 작은 혈관을 이용하여 여러 실험 조건에서 장력의 변화를 측정하여 다음과 같은 결과를 얻었다. 고려홍삼 사포닌성분은 농도 의존적으로 (0.01 mg/ml ~ 1 mg/ml) 혈관 평활근을 이완시켰으며 내피세포를 제거한 상태에서도 혈관의 이완효과는 지속되었다. A23187 이나 phorbol 12,13-dibutyrate에 의한 수축에서는 고려홍삼 사포닌에 의한 혈관의 이완효과가 나타나지 않았다. 고려홍삼 사포닌에 의한 혈관이완효과는 실험용액의 K^+ 농도를 증가시키면 감소되었으며 각종 K^+ 이온통로 억제제인 tetraethylammonium, glybenclamide, 4-aminopyridine 및 $BaCl_2$를 전처치한 결과 $BaCl_2$에 의해서만 농도에 의존적으로 고려홍삼 사포닌에 의한 혈관이완작용이 억제되었다. 이상의 실험결과로부터 고려홍삼 사포닌은 장간막 동맥의 저항혈관에서 K^+의 유출을 증가시켜 혈관평활근을 이완시키며 Ba^{2+}에 의하여 차단되는 K^+ 이온통로가 고려홍삼 사포닌에 의한 혈관이완작용에 관여함을 알 수 있었다.

REFERENCE

1. Kang, S. Y. and Kim, N. D. : *Korean J. Ginseng Sci.* **16,** 175 (1992).
2. Kim, N. D., Kim, S. H., Kang, K. W. and Choi, K. J. : *Korean J. Ginseng Sci.* **21,** 119 (1997).
3. Chen, X., Gillis, C. N. and Moalli, R. : *Br. J. Pharmacol.* **82,** 485 (1984).
4. Kim, N. D., Kang, S. Y. and Schini-Kerth, V. B. : *Gen. Pharmacol.* **25,** 1071 (1994).
5. Kim, N. D., Kang, S.Y., Park, J. H. and Schini-Kerth, V. B. : *Eur. J. Pharmacol.* **367,** 41 (1999).
6. Kim, N. D., Kang, S. Y., Kim, M. J., Park, J. H. and Schini-Kerth, V. B. : *Eur. J. Pharmacol.* **367,** 51 (1999).
7. Li, Z., Chen, X,, Niwa, Y., Sakamoto, S. and Nakaya Y. J. : *Cardiovasc. Pharmacol.* **37,** 41 (2001).
8. Nelson, M. T. and Quayle, J. M. : *Am. J. Physiol.* **268,** C799 (1995).
9. Chang, S. J., Suh, J. S., Jeon, B. H., Nam, K. Y. and Park, H. K. : *Korean J. Ginseng Sci.* **18,** 95 (1994).
10. Nishimura, J., Khalil, R. A., Drenth, J. P., van Breemen, C. : *Am. J. Physiol.* **259,** H2 (1990).
11. Nah, S. Y., Park, H. J. and McCleskey, E. W. : *Proc. Natl. Acad. Sci.* **92,** 8739 (1995).
12. Wang, X. M., Qi. Y., Sun, C. W., Zhong, G. C., Jiang, Y. and Qiu, Y. H. : *Chung Kuo Chung Yao Tsa Chih* **19,** 621 (1994).
13. Brayden, J. E. and Nelson, M. T. : *Science* **256,** 532 (1992).
14. Beech, D. J. and Bolton, T. B. : *J. Physiol.* **418,** 293 (1989).
15. Standen, N. B., Quayle, J. M., Davies, N. W., Brayden, J. E., Huang, Y. and Nelson, M. T. : *Science* **245,** 177 (1989),
16. Edwards, F. R. and Hirst, G. D. : *J. Physiol.* **404,** 437 (1988).
17. Asano, M., Masuzawa-Ito, K., Matsuda, T., Suzuki, Y., Oyama, H., Shibuya, M. and Sugita, K. : *J. Pharmacol. Exp. Ther.* **267,** 1277 (1993).
18. Yuan, X. J., Goldman, W. F., Tod, M. L., Rubin, L. J., Blaustein, M. P. : *Am. J. Physiol.* **264,** L107 (1993).
19. Xu, C., Lu, Y., Tang, G. and Wang, R. : *Am. J. Physiol.* **277,** G1055 (1999).
20. Quayle, J. M., McCarron, J. G., Brayden, J. E. and Nelson, M. T. : *Am. J. Physiol.* **265,** C1363 (1993).
21. Standen, N. B. and Stanfield, P. R. : *J. Physiol.* **280,** 169 (1978).