Possible Involvement of K^+ Channel Opening to the Interleukin-1β-Induced Inhibition of Vascular Smooth Muscle Contraction

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ABSTRACT. We have previously shown that interleukin-1β relaxes vascular smooth muscle by the NO-dependent and independent mechanisms (Takizawa *et al.*: Eur. J. Pharmacol. 330: 143–150, 1997). In this study, we investigated the mechanism of NO-independent relaxation. Treatment of the rat aorta with interleukin-1β for 24 hr inhibited the high-K^+ induced contraction by decreasing cytosolic Ca^{2+} level ($[Ca^{2+}]_i$). The relationship between $[Ca^{2+}]_i$ and tension in intact muscle and the pCa-tension curves in permeabilized muscle suggested that Ca^{2+} sensitivity of contractile element was not changed after the interleukin-1β-treatment. After a treatment with interleukin-1β for 24 hr, contractile effects of phenylephrine (1 μM–10 μM) were markedly inhibited in the presence of L-NMMA (100 μM) applied to inhibit NO synthesis. A blocker of ATP-sensitive K^+ channel, glibenclamide (1 μM), partially recovered the interleukin-1β-induced inhibition. In contrast, a blocker of Ca^{2+}-activated K^+ channel, charybdotoxin (0.1 μM), was ineffective. These results suggest that membrane hyperpolarization due to activation of ATP-sensitive K^+ channels may partly be responsible for the NO-independent mechanism of interleukin-1β-induced inhibition of vascular smooth muscle contraction.—KEY WORDS: interleukin-1β, K^+ channel, relaxation, vascular smooth muscle.

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In septic shock, bacterial lipopolysaccharide stimulates macrophages, monocytes, endothelial cells and vascular smooth muscle cells to release interleukin-1β [5]. Prolonged exposure to interleukin-1β decreases vascular tone and causes systematic vasodilation [1, 11]. Since interleukin-1β stimulates the expression of inducible form of NO synthase in the vascular smooth muscle cells which generates large quantities of NO over prolonged period, the vasodilatory effect of interleukin-1β has been suggested to be mediated by NO [2, 3, 6].

Our recent study showed that phenylephrine-induced contraction was inhibited by *in vitro* pre-incubation of the rat aorta with interleukin-1β [1, 6, 15]. Analyzing the mechanisms of interleukin-1β-induced inhibition, we have shown that interleukin-1β-induced vasodilation was mediated not only by NO-dependent mechanism but also by NO-independent mechanism(s) [15]. The purpose of the present study was to clarify the mechanisms of the NO-independent vascular relaxation after the prolonged treatment with interleukin-1β.

MATERIALS AND METHODS

Experiments in intact tissue: Male Wistar rats (200–250 g) were killed by a sharp blow on the neck and exsanguination. The thoracic aorta was isolated, placed in Hanks solution and cut into approximately 4 mm × 8 mm strips with sterilized instruments. The mesenteric artery was also isolated. The endothelium was removed by rubbing with a stainless steel rod. Strips were then placed in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 400 μM L-arginine with 20 *n*g/m*l* interleukin-1β for 24 hr in the CO_2 incubator at 37°C (interleukin-1β-aorta). Other strips were treated with similar DMEM solution but not containing interleukin-1β for 24 hr (control-aorta). Concentration of endotoxin in DMEM, measured by Toxicolor System (Seikagaku Kogyo), was less than 150 *p*g/m*l*, a concentration that does not inhibit the contraction in the rat aorta [9].

After treatment with interleukin-1β, muscle strips were placed in normal solution, which contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, ethylenediaminetetraacetic acid (EDTA) 0.01 and glucose 5.5. This solution was saturated with 95% O_2–5% CO_2 mixture at 37°C and pH 7.4.

Muscle tension was recorded isometrically with a force-displacement transducer. Each muscle strip was attached to a holder under the resting tension of 10 mN and equilibrated for about 120 min until the contractile response to high KCl became stable. High KCl solution was made by replacing NaCl with equimolar KCl in normal solution. At the end of tension measurement, wet weight of each muscle strips was measured. Contractile force is shown by the absolute force (mN) per mg wet weight of tissue.

Cytosolic Ca^{2+} level ($[Ca^{2+}]_i$) was measured as described by Ozaki *et al.* [12] and Sato *et al.* [14] with a fluorescent Ca^{2+} indicator Fura-PE3. Muscle strips were treated with acetoxymethyl ester of Fura-PE3 (5 μM) containing 0.2% cremophor EL at room temperature for about 6 hr.

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Experiments in α-toxin treated tissue: Mesenteric artery was isolated from rats, placed in Hanks solution and cut into approximately 0.5 mm × 2 mm strips with sterilized instruments. After treatment with interleukin-1β, muscle strips were placed in relaxing solution, which contained (mM): 2 mM Mg methansulphonate, 5 mM Mg-ATP, 2 mM O,O'-Bis (2-aminoethyl) ethyleneglycol-*N,N,N',N'* tetraacetic acid (EGTA), 20 mM piperazine-*N,N'*-bis (2-ethanesulfonic acid) (PIPES), 2 mM creatine phosphate, 1 μM carbonyl cyanide p-trifluoro-methoxy-phenyldrazone (FCCP), 1 μM E-64. The pH of this solution was adjusted to 6.8 (25°C) by adding KOH, and ion strength was 0.2. The Ca^{2+} buffered solution was prepared by adding Ca methansulphonate. Muscle tension was recorded isometrically with a force-displacement transducer. Each muscle strip was attached to a holder under the resting tension of 1 mN and equilibrated for about 20 min until the contractile response to 3 μM Ca^{2+} buffering solution became stable. Contractile force is shown by the absolute force (mN) per mm width of tissue.

Chemicals: The chemicals used were interleukin-1β (Genzyme), DMEM (Nissui Pharmaceutical), fetal bovine serum (Hyclone), phenylephrine hydrochloride, (Sigma Chemical), EDTA, (Dojindo Laboratories), phentolamine (Ciba-Geigy Japan), L-NMMA, tetraethylammonium (Wako Pure Chemicals).

Statistics: The results of the experiments are expressed as mean ± S.E.M. Unpaired Student's *t*-test was used for statistical analysis of the results and $P<0.05$ was taken as significant.

RESULTS

Effects of treatment with interleukin-1β on $[Ca^{2+}]_i$ and contraction of rat aorta: Cumulative addition of high K^+ (15.4–72.7 mM) induced graded increases in $[Ca^{2+}]_i$ and muscle tension in a concentration-dependent manner. The treatment with interleukin-1β inhibited the increases in both $[Ca^{2+}]_i$ and muscle tension due to high K^+ (Fig. 1A and B).

$[Ca^{2+}]_i$-tension relationship: $[Ca^{2+}]_i$-tension relationship was constructed by the data of Fig. 1A and B. As shown in 2A, the $[Ca^{2+}]_i$-tension relationship is not different between the control and interleukin-1β aortas.

In α-toxin-permeabilized rat mesenteric artery, cumulative addition of Ca^{2+} induced concentration-dependent contractions. There was no difference between pCa^{2+}-tension curves of the control and interleukin-1β-mesenteric arteries (Fig. 2B).

These results suggest that the inhibition of of vascular smooth muscle contraction in interleukin-1β treated tissue is not attributable to the decreased Ca^{2+}-sensitivity of contractile elements, but the decrease in $[Ca^{2+}]_i$ is the cause of inhibition of contraction.

The effects of the selective K^+ channel modulator on phenylephrine-induced contraction: As shown in Fig. 3, cumulative addition of phenylephrine caused a concentration-dependent contraction in the control-aorta. In the interleukin-1β-aorta, the phenylephrine-induced contractions were markedly attenuated in the presence of L-NMMA, an inhibitor of NO synthase. A selective Ca^{2+} activated K^+ channel blocker, charybdotoxin (0.1 μM), was added 30 min before cumulative addition of phenylephrine. Charybdotoxin did not affect the phenylephrine-induced contraction in the interleukin-1β-aorta (Fig. 3). On the other hand, 1 μM glibenclamide which maximally inhibits ATP sensitive K^+ channel [4, 13], significantly augmented the maximum force of the interleukin-1β-aorta from 0.6 ± 0.1 (n=13) to 1.3 ± 0.3 (n=14) mN/mg wet weight ($P<0.05$, Fig. 4).

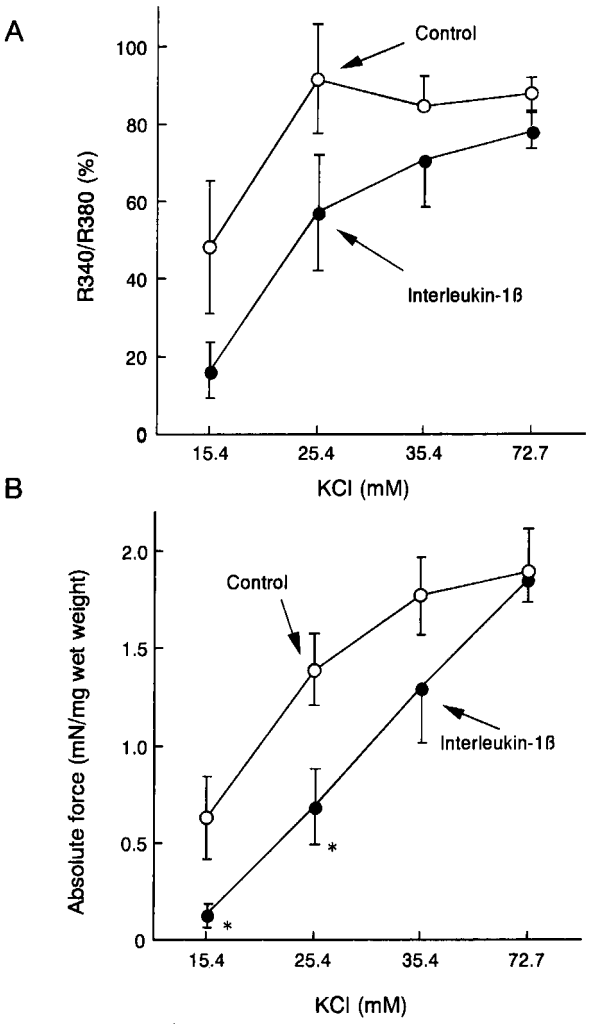

Fig. 1. Change in cytosolic Ca^{2+} level (A) and muscle tension (B) induced by high K^+ in rat aorta treated with (●: interleukin-1β) or without (○: control) interleukin-1β (20 *ng/ml*) for 24 hr. High K^+ (15.4–72.7 mM) was cumulatively applied to the muscle strips. * Significantly different with $P<0.05$. Each point represents mean of 6–7 strips and S.E.M. is shown by vertical and horizontal bar.

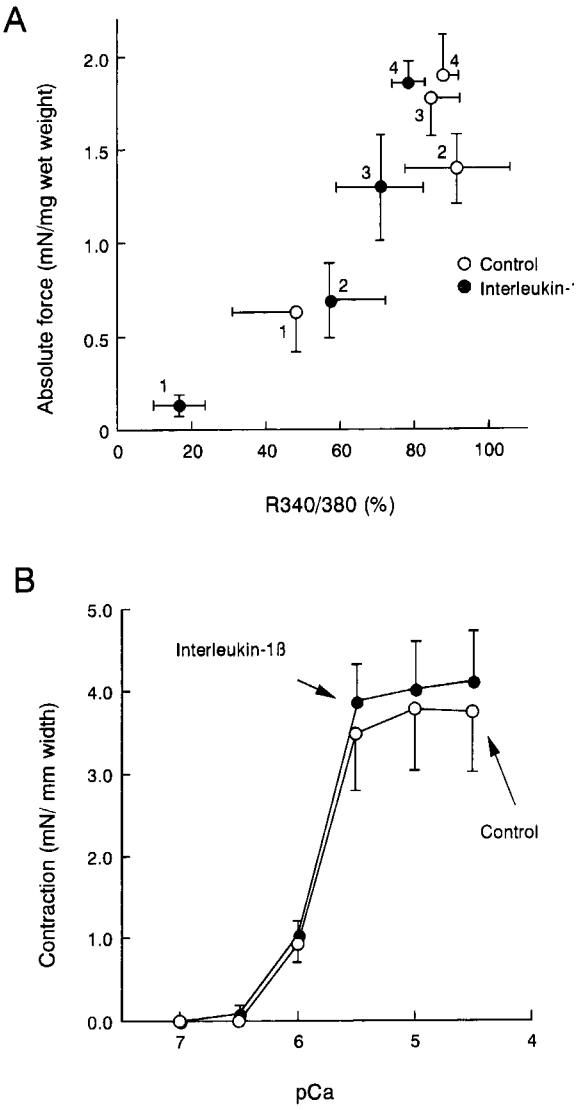

Fig. 2. A: The relationship between $[Ca^{2+}]_i$ and the muscle contraction in rat aorta treated with (●: interleukin-1β) or without (○: control) interleukin-1β (20 *n*g/m*l*) for 24 hr. Data was adapted from Fig. 1A and B. Numbers (1–4) attached to the data points indicate the concentration of KCl (1. 15.4 mM, 2. 25.4 mM, 3. 35.4 mM, 4. 72.7 mM). B: Contraction of α-toxin permeabilized rat mesenteric artery treated with (●: interleukin-1β) or without (○: control) interleukin-1β (20 *n*g/m*l*) for 24 hr. Ca^{2+} was cumulatively applied to the permeabilized muscle strips. Each point represents mean of 4–6 strips and S.E.M. is shown by vertical bar.

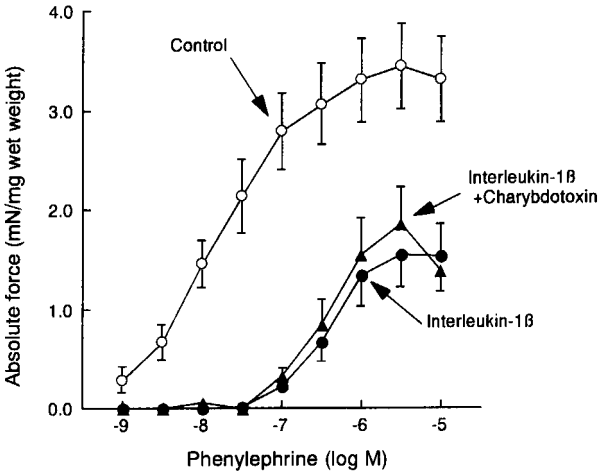

Fig. 3. The effect of charybdotoxin on contraction in the rat aorta after a 24 hr treatment with (●: interleukin-1β) or without (○: control) interleukin-1β. In order to inhibit the possible effects of NO on Ca^{2+}-activated K^+ channel [8], experiments were conducted in the presence of L-NMMA (100 μM). Phenylephrine was cumulatively applied to the muscle strips. Charybdotoxin (0.1 μM, ▲) together with L-NMMA (100 μM) was applied to normal solution 30 min before addition of phenylephrine. Each point represents mean of 5 strips and S.E.M. is shown by vertical bar. * Significantly different from interleukin-1β treated muscle with $P<0.05$.

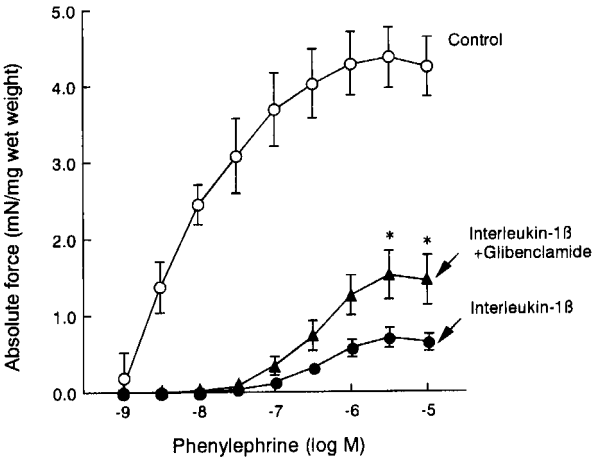

Fig. 4. The effect of glibenclamide on contraction in the rat aorta after a 24 hr treatment with (●: interleukin-1β) or without (○: control) interleukin-1β. Phenylephrine was cumulatively applied to the muscle strips. Glibenclamide (1 μM, ▲) was applied to normal solution 30 min before addition of phenylephrine. Each point represents mean of 5 strips and S.E.M. is shown by vertical bar. * Significantly different from interleukin-1β treated muscle with $P<0.05$.

DISCUSSION

As demonstrated in Fig. 1A and B, treatment of vascular tissue with interleukin-1β inhibited the increases in $[Ca^{2+}]_i$ and contraction elicited by high K^+ solutions. The $[Ca^{2+}]_i$-tension curve in the interleukin-1β-aorta obtained in the presence of different concentrations of KCl was not different from that in the control-aorta. Furthermore, Ca^{2+}-induced contraction of α-toxin permeabilized rat mesenteric artery was not affected by the pretreatment with interleukin-1β. These results suggest that interleukin-1β does not affect the

Ca^{2+} sensitivity of aortic smooth muscle and decrease in $[Ca^{2+}]_i$ is responsible for the decreased contractility of vascular smooth muscle. Since NO inhibits Ca^{2+} sensitivity of smooth muscle contractile elements through cGMP-dependent mechanism [7], it is suggested that the inhibition by interleukin-1β of the high K^+-induced contraction arises mainly through a cGMP-independent mechanism.

We have previously found that phenylephrine-induced contraction is inhibited by the pretreatment with interleukin-1β, and this inhibition was reversed by membrane depolarization with 25.4 mM K^+ or by block of K^+ channels with tetraethylammonium [15]. These findings suggest that the treatment with interleukin-1β can activate K^+ channels to hyperpolarize the membrane potential, resulting in a reduction of Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Several types of selective K^+ channel blockers have been developed, and widely used to identify the subtypes of K^+ channel including Ca^{2+} activated K^+ channel, ATP sensitive K^+ channel and delayed rectifier K^+ channel [7, 9]. Charybdotoxin is a selective blocker of Ca^{2+}-activated K^+ channel. In the present study, the contraction of interleukin-1β-aorta was not affected by charybdotoxin in the presence of L-NMMA (100 μM). In contrast, an ATP-sensitive K^+ channel blocker, glibenclamide, partially augmented the contraction of the interleukin-1β-aorta. Furthermore, non-selective K^+ channel blocker, tetraethylammonium restored the contraction [15]. These results suggest that interleukin-1β opens ATP-sensitive K^+ channel and inhibits the contraction.

In conclusion, the pretreatment with interleukin-1β for 24 hr may open ATP-sensitive K^+ channel and inhibit contraction of vascular smooth muscle by hyperpolarization of membrane potential which may partly responsible for the NO-independent mechanism of the interleukin-1β-induced inhibition. Possible involvement of other types of K^+ channels remains to be elucidated.

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