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Interleukin-1β-induced, nitric oxide-dependent and -independent inhibition of vascular smooth muscle contraction

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Abstract

Stimulation of vascular smooth muscle by bacterial lipopolysaccharide has been shown to produce interleukin-1β and to induce vasodilation in septic shock. To understand the mechanisms of interleukin-1β-induced relaxation, we examined the effects of interleukin-1β on contractility and cyclic GMP contents of vascular smooth muscle. After treatment of the rat aorta with interleukin-1β (20 ng/ml) for 6 h, the cyclic GMP content increased and the contraction induced by phenylephrine (1 μM) was partially inhibited. An inhibitor of nitric oxide (NO) synthase, N^G-monomethyl-L-arginine (L-NMMA, 100 μM), prevented the inhibitory effect of interleukin-1β. After treatment with interleukin-1β for 24 h, the phenylephrine-induced contraction was inhibited more strongly. Neither L-NMMA (100 μM) nor aminoguanidine (100 μM) reversed the inhibition, whereas methylene blue (10 μM) partially reversed the inhibition. After treatment with interleukin-1β for 12 or 24 h, the cyclic GMP content increased but to a level lower than that obtained with a 6-h treatment. The effects of sodium nitroprusside (1 μM) to inhibit the phenylephrine-induced contraction and to increase the cyclic GMP content were markedly suppressed by the 24-h interleukin-1β treatment. In contrast, the 24-h interleukin-1β treatment did not change the ability of 8-bromo-cGMP to relax the phenylephrine-stimulated aorta. Addition of L-NMMA (1 mM) during the 24 h treatment prevented NO production and preserved the sodium nitroprusside-induced cGMP generation by interleukin-1β. The 24 h interleukin-1β treatment increased the threshold concentration of KCl needed to induce contraction without changing the maximum contraction. In the presence of 25.4 mM KCl or the non-selective K^+ channel inhibitor, tetraethylammonium, the inhibitory effect of the 24-h interleukin-1β treatment on phenylephrine-induced contraction was restored. These results suggest that interleukin-1β inhibits vascular smooth muscle contraction by a time-dependent, dual mechanism. After a 6-h treatment with interleukin-1β, the NO/cyclic GMP system is activated. After a 24-h interleukin-1β treatment, in contrast, the NO/cyclic GMP system may be desensitized and the contraction of vascular smooth muscle is inhibited by another mechanism, possibly membrane hyperpolarization. © 1997 Elsevier Science B.V.

Keywords: Aorta, rat; cGMP; Relaxation; Nitric oxide (NO) synthase

1. Introduction

In septic shock, bacterial lipopolysaccharide stimulates macrophages, monocytes, endothelial cells and vascular smooth muscle cells to synthesize interleukin-1β (Dinarello, 1988). Patients with septic syndrome have elevated levels of circulatory interleukin-1, interleukin-6, lipopolysaccharide and tumor necrosis factor-α (Casey et al., 1993). Prolonged exposure of blood vessels to interleukin-1β decreases vascular tone and causes systemic vasodilation (Beasley et al., 1989; Okusawa et al., 1988).

The effect of interleukin-1β on vascular smooth muscle has been suggested to be mediated by nitric oxide (NO) (French et al., 1991; Beasley et al., 1991). Interleukin-1β activates the synthesis of inducible nitric oxide synthase which usually does not exist in vascular smooth muscle cells. The inducible NO synthase in vascular smooth muscle is the same type as that induced in macrophages and does not require Ca^{2+} and calmodulin for its regulation (Busse and Mulsch, 1990; Kanno et al., 1993). Once this enzyme is induced, a large amount of NO is produced. When NO reaches vascular smooth muscle cells, guanylate cyclase is activated to synthesize cyclic GMP (cGMP), which relaxes vascular smooth muscle through mechanisms including membrane hyperpolarization, inhibition of phosphatidic inositol turnover and inhibition of contractile elements (Karaki et al., 1988). Supporting this hypothesis, NO synthase inhibitors have been suggested to be effective therapeutic drugs for human septic shock (Petros et al.,

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1991). However, there are some reports showing that NO synthase inhibitors cannot completely reverse the hypotensive effect of lipopolysaccharide (Nava et al., 1991; Evans et al., 1994; Wolfe and Dasta, 1995), suggesting that there is an additional mechanism for septic shock and the effect of interleukin-1β.

To further understand the mechanism of interleukin-1β-induced vasodilation, we treated isolated rat blood vessels with interleukin-1β for up to 24 h and measured the changes in smooth muscle contractility and cGMP content. Results indicated that not only the NO/cGMP system but also an additional mechanism are responsible for the interleukin-1β-induced vasodilation.

2. Materials and methods

2.1. Preparations and treatment

Male Wistar rats (200–250 g) were killed by a sharp blow on the neck and exsanguination. The thoracic aorta was isolated and cut into approximately 4 mm wide 8 mm long strips in Hanks' solution with sterilized instruments. The vascular 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 ng/ml interleukin-1β for 6, 12 and 24 h in CO_2 incubator at 37°C (6-h interleukin-1β, 12-h interleukin-1β and 24-h interleukin-1β, respectively). Other strips were treated with a similar DMEM solution but not containing interleukin-1β for 6, 12 and 24 h (6-h control, 12-h control and 24-h control, respectively). The concentration of endotoxin in DMEM, measured by using the Toxicolor System (Seikagaku Kogyo, Japan), was less than 50 pg/ml, a concentration that does not inhibit contraction of the rat aorta (Nakaki et al., 1992). In some experiments, 1 mM N^G-monomethyl-L-arginine (L-NMMA) was added to DMEM.

2.2. Muscle tension and solutions

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 a 95% O_2/5% CO_2 mixture at 37°C and pH 7.4. In some experiments, 100 μM L-NMMA was added to the normal solution.

Muscle tension was recorded isometrically with a force-displacement transducer. Each muscle strip was attached to a holder under 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 measurements, the wet weight of each muscle strips was measured. Contractile force is shown in mN/mg wet weight of tissue.

2.3. cGMP content

cGMP content in the aorta was measured with an enzyme-immunoassay. Subsequent to treatment with interleukin-1β, muscle strips weighing 5–13 mg were stabilized in normal solution for 120 min. The strips treated with or without interleukin-1β for 24 h were divided into a resting group, a sodium nitroprusside group and a L-NMMA group. Strips in the sodium nitroprusside group and L-NMMA group were treated with 1 μM sodium nitroprusside for 5 min and 100 μM L-NMMA for 50 min, respectively. An inhibitor of phosphodiesterase, M&B 22,948 (10 μM), was added 20 min before the end of the treatment. At the end of the treatment with sodium nitroprusside or L-NMMA, muscle strips were frozen in liquid nitrogen and homogenized in normal solution containing 10% trichloroacetic acid. After centrifugation at 1500 r.p.m., trichloroacetic acid in the supernatant was removed by washing with water-saturated ether, and cGMP was assayed with an enzyme-immunoassay kit (Cayman, Ann Arbor, MI, USA).

2.4. NO_2^- content

NO is a free radical which is easily metabolized to NO_2^-. In the present experiments, the NO content of DMEM was measured by detecting the 12-h or 24-h accumulation of NO_2^- with 2,3-diaminonaphthalene as reported by Wiersma (1970) and Misko et al. (1993). 2,3-Diaminonaphthalene (0.5 μg dissolved in 10 μl 0.62 M HCl) and 100 μl samples were mixed and placed in a glass tube in a dark room. After 15 min, 2.8 M NaOH (5 μl) and water (900 μl) were added to the tube and mixed. Fluorescence was measured at an excitation of 365 nm and an emission of 450 nm. NO_2^- content was calculated from a standard curve. In a preliminary experiment, we confirmed that the detection limit of this method is approximately 30 nM NO.

2.5. Chemicals

The chemicals used were interleukin-1β (Genzyme, USA), DMEM (Nissui Pharmaceutical, Japan), fetal bovine serum (Cell Culture Laboratories, USA), phenylephrine, phentolamine, 8-bromo-cGMP (Sigma, St. Louis, MO, USA), EDTA, 2,3-diaminonaphthalene (Dojindo Laboratories, Japan), L-NMMA, methylene blue and sodium nitroprusside (Wako, Japan).

2.6. Statistics

The results of the experiments are expressed as means ± S.E.M. Unpaired Student's *t*-test was used for statistical

analysis of the results and $P < 0.05$ was taken as significant.

3. Results

3.1. Effects of treatment with interleukin-1β on contraction of rat aorta

In the 6-h control aorta, cumulative addition of phenylephrine induced concentration-dependent contraction with a threshold concentration of 3 nM, an EC_{50} of 26.2 nM and a maximum force of 3.6 mN/mg wet weight. In the 6-h interleukin-1β aorta, the contractile effect of phenylephrine was inhibited, increasing the threshold concentration and the EC_{50} to 30 nM and 200 nM, respectively, and decreasing the maximum force to 61.1% of the 6-h control. When contraction was measured in the presence of L-NMMA, the EC_{50} of the 6-h control aorta was decreased to 10.4 nM without changing the threshold and maximum force. L-NMMA restored the phenylephrine-induced contraction of the 6-h interleukin-1β aorta, decreasing the threshold concentration and the EC_{50} to 10 nM and 54.0 nM, respectively, and increased the maximum force to 125% of the 6-h control aorta (Fig. 1).

In the 24-h control aorta, cumulative addition of phenylephrine induced contraction with a threshold concentration of 1 nM, an EC_{50} of 14.0 nM and maximum force of 3.3 mN/mg wet weight. In the 24-h interleukin-1β aorta, the contractile effect of phenylephrine was inhibited, increasing the threshold concentration and the EC_{50} to 100 nM and 357 nM, respectively, and decreasing the maximum force to 18.2% of the 24-h control aorta. Addition of L-NMMA (100 μM) during tension measurement decreased the EC_{50} to 6.5 nM without changing the threshold concentration or maximum force of the 24-h control aorta. L-NMMA partially restored the phenylephrine-induced

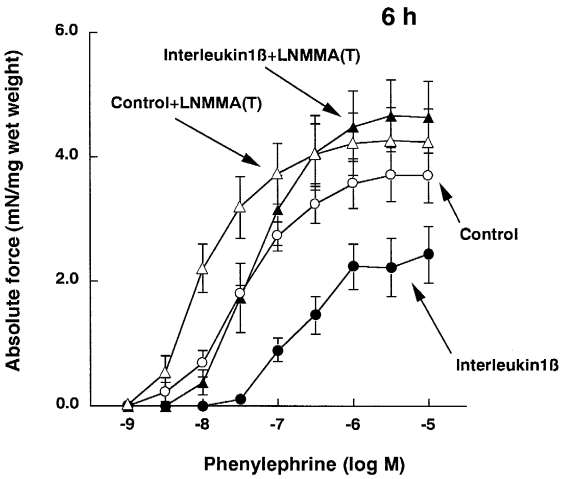

Fig. 1. Phenylephrine-induced contraction in the rat aorta after a 6-h treatment with interleukin-1β. Phenylephrine was cumulatively added to muscle strips treated with DMEM for 6 h in the absence (control) or in the presence of 20 ng/ml interleukin-1β. LNMMA(T): L-NMMA (100 μM) was added 30 min before the addition of phenylephrine. Each point represents the mean for 6–8 strips and S.E.M. is shown by vertical bar.

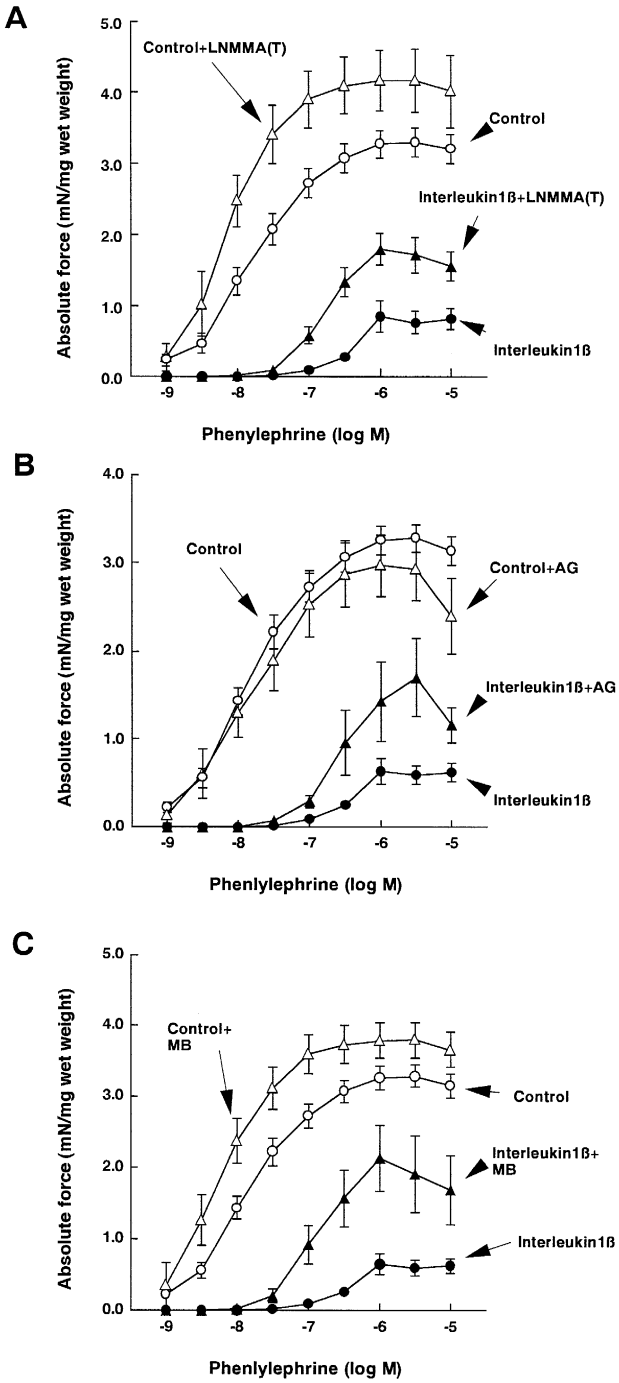

Fig. 2. Phenylephrine-induced contraction in the rat aorta after a 24-h treatment with interleukin-1β and the effect of NO synthase inhibitors. Phenylephrine was cumulatively added to muscle strips after treatment with DMEM for 24 h in the absence (control) or in the presence of 20 ng/ml interleukin-1β. (A) LNMMA(T): L-NMMA (100 μM) was added 30 min before the addition of phenylephrine. (B) AG: aminoguanidine (100 μM) was added 30 min before the addition of phenylephrine. (C) MB: methylene blue (10 μM) was added 30 min before the addition of phenylephrine. Each point represents the mean for 5–31 strips and S.E.M. is shown by vertical bar.

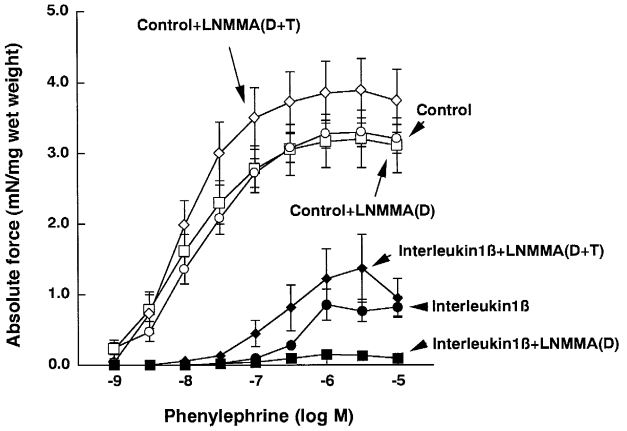

Fig. 3. Phenylephrine-induced contraction in the rat aorta after a 24-h treatment with interleukin-1β and L-NMMA. Phenylephrine was cumulatively added to the muscle strips after treatment with DMEM for 24 h in the absence (control) or in the presence of 20 ng/ml interleukin-1β. LNMMA(D): L-NMMA (1 mM) was added to DMEM for treatment with or without interleukin-1β. LNMMA(D + T): L-NMMA (1 mM) was added to DMEM and L-NMMA (100 μM) was also added to the solution for tension measurements 30 min before the addition of phenylephrine. Each point represents the mean for 5–20 strips and S.E.M. is shown by vertical bar.

maximum contraction of the 24-h interleukin-1β aorta to 54.5% of the 24-h control aorta (Fig. 2A). The selective inhibitor of inducible NO synthase, aminoguanidine (100 μM), did not restore the phenylephrine-induced contraction of the 24-h interleukin-1β aorta (Fig. 2B). The inhibitor of guanylate cyclase, methylene blue (10 μM), restored the phenylephrine-induced contraction of the 24-h interleukin-1β aorta, although the recovery was partial (EC_{50} was 73.6 μM and maximum contraction was 63.6% of the 24-h control aorta; Fig. 2C).

In the 24-h control aorta, addition of L-NMMA to both DMEM and the solution for tension measurement did not affect the phenylephrine-induced contraction (Fig. 3). In the 24-h interleukin-1β aorta, addition of L-NMMA to DMEM but not to the solution for tension measurements

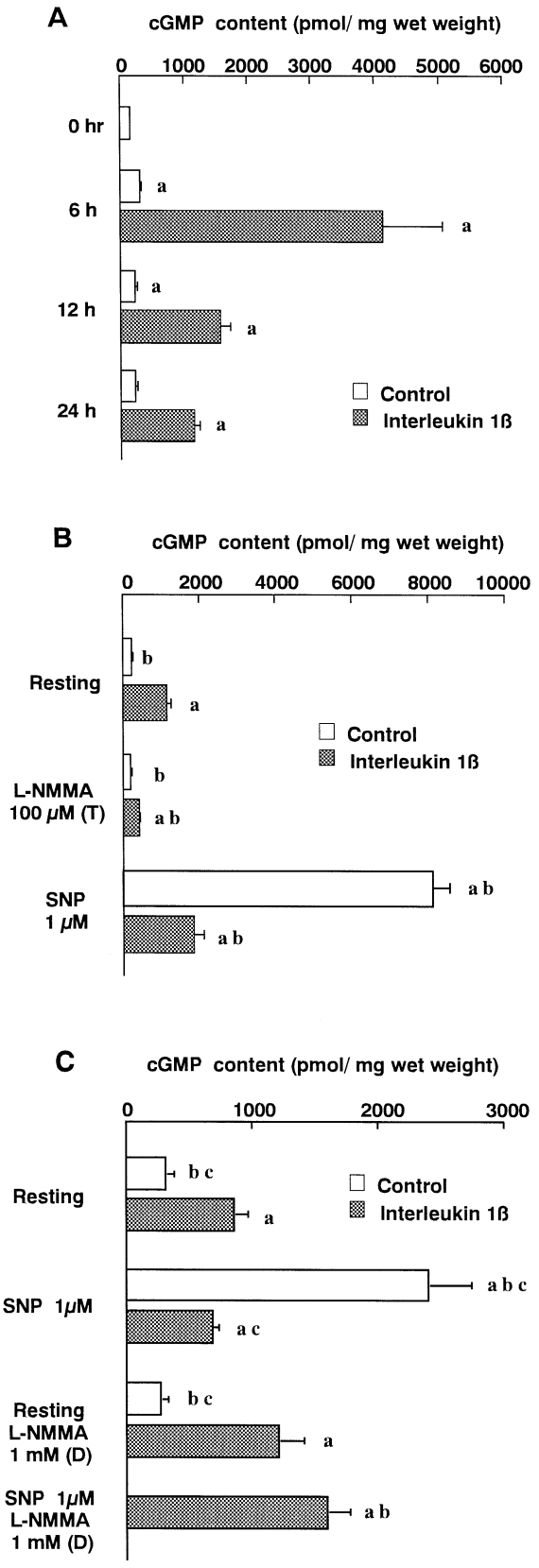

Fig. 4. Change in cyclic GMP content in the rat aorta treated with interleukin-1β. (A) Muscle strips were pretreated without (open columns) or with interleukin-1β (20 ng/ml, hatched columns) for 0, 6, 12 and 24 h. [a] $P < 0.05$: significantly different from freshly isolated muscle strips (0 h). Each point represents the mean for 4–7 muscle strips and S.E.M. is shown by horizontal bar. (B) Muscle strips were pretreated without (open columns) or with interleukin-1β (20 ng/ml, hatched columns) for 24 h. Muscle strips were incubated with L-NMMA (100 μM, T) or sodium nitroprusside (1 μM) for 50 min or 5 min, respectively. [a] $P < 0.05$: significantly different from muscle strips treated without interleukin-1β. [b] $P < 0.05$: significantly different from muscle strips treated with interleukin-1β. Each point represents the mean for 5–7 muscle strips and S.E.M. is shown by horizontal bar. (C) Muscle strips were pretreated without (open columns) or with interleukin-1β (20 ng/ml, hatched columns) for 24 h. Muscle strips were treated with (D) or without L-NMMA (1 mM) for 24 h. After pretreatment for 24 h, muscle strips were incubated with sodium nitroprusside (1 μM) for 5 min. [a] $P < 0.05$: significantly different from muscle strips treated without interleukin-1β. [b] $P < 0.05$: significantly different from muscle strips treated with interleukin-1β. [c] $P < 0.05$: significantly different from muscle strips treated with interleukin-1β and L-NMMA. Each point represents the mean for 4–5 muscle strips and S.E.M. is shown by horizontal bar.

induced additional inhibition of the phenylephrine-induced contractions increasing the EC_{50} to 1.0 mM, and inhibited the maximum force to 0.1 mN/mg wet weight. However, addition of L-NMMA to both DMEM and the solution for tension measurements partially restored the contractile effect of phenylephrine in the 24-h interleukin-1β aorta by decreasing the EC_{50} to 138 nM and increasing the maximum force to 1.2 mN/mg wet weight (Fig. 3).

3.2. NO_2^- content in DMEM

The amount of NO_2^- accumulated in DMEM in 6 h or 24 h in control and 6 h in the presence of interleukin-1β was below the detection limit (approximately 10 nmol/mg wet weight). The NO_2^- accumulated in 24 h in the presence of interleukin-1β was 34.8 ± 3.1 nmol/mg wet weight ($n = 4$). When L-NMMA (1 mM) was added to the DMEM, the NO_2^- content decreased to 12.7 ± 2.1 nmol/mg wet weight ($n = 4$, $P > 0.05$).

3.3. The effects of interleukin-1β on the cGMP-mediated relaxation

In the 6-h control and 24-h control aortas, sodium nitroprusside (1 μM) inhibited the contraction induced by phenylephrine (1 μM) almost completely (6 h: $97.6 \pm 1.6\%$, $n = 8$, 24 h: $99.3 \pm 0.4\%$, $n = 4$). In the 6-h interleukin-1β aorta, sodium nitroprusside (1 μM) also inhibited the contraction although the inhibitory effect was significantly smaller than that in the 6-h control aorta. ($46.5 \pm 14.5\%$, $n = 8$, $P < 0.05$). In the 24-h interleukin-1β aorta, sodium nitroprusside (1 μM) did not relax the phenylephrine-induced contraction ($n = 4$).

A membrane-permeable cGMP analogue, 8-bromo-cGMP (100 μM), inhibited the phenylephrine (1 μM)-induced contraction in the 24-h control and 24-h interleukin-1β aortas by $70.0 \pm 8.8\%$ ($n = 9$) and $72.4 \pm 5.3\%$ ($n = 9$, $P > 0.05$), respectively.

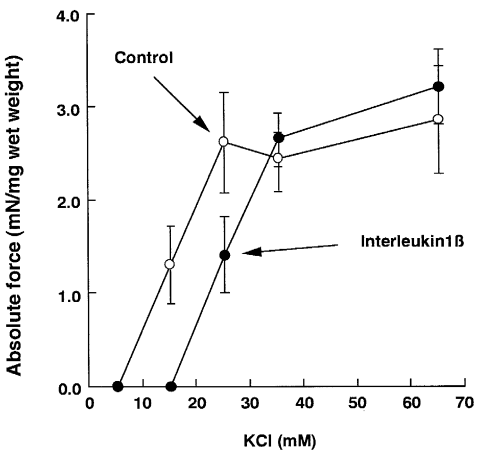

Fig. 5. High-K^+-induced contraction in the rat aorta after a 24-h treatment with interleukin-1β. KCl was cumulatively added to muscle strips treated with DMEM for 24 h in the absence (control) or in the presence of 20 ng/ml interleukin-1β. Each point represents the mean for 5 strips and S.E.M. is shown by vertical bar.

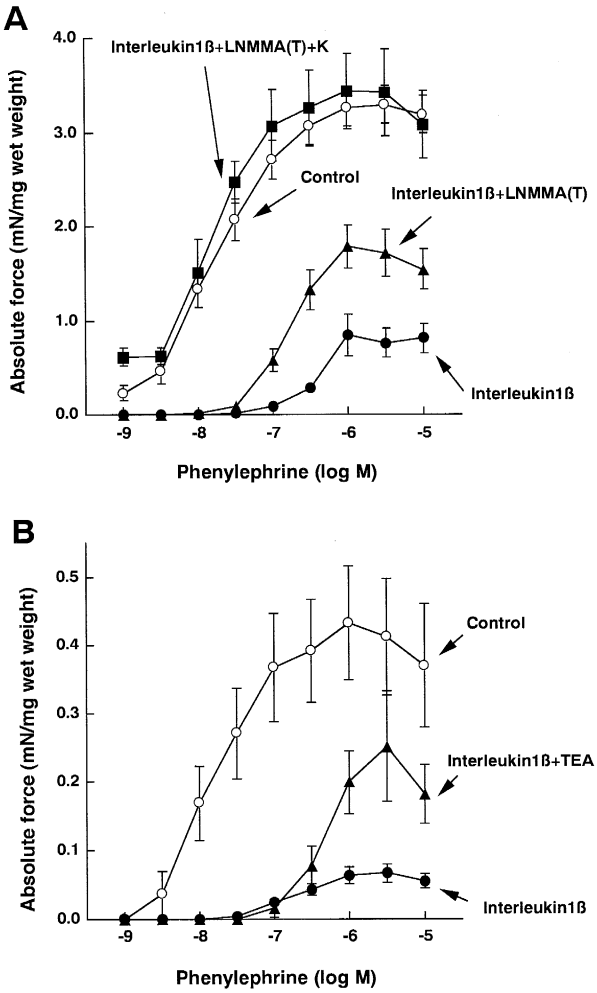

Fig. 6. Effect of depolarization of the membrane potential on the phenylephrine-induced contraction in the rat aorta treated with interleukin-1β. Phenylephrine was cumulatively added to muscle strips after treatment with DMEM for 24 h in the absence (control) or in the presence of 20 ng/ml interleukin-1β. (A) LNMMA(T): L-NMMA (100 μM) was added 30 min before the addition of phenylephrine. LNMMA(T) + K: L-NMMA (100 μM) and 25.4 mM KCl were added 30 min before the addition of phenylephrine. Each point represents the mean for 5–20 muscle strips and S.E.M. is shown by vertical bar. (B) TEA: tetraethylammonium (30 mM) was added 30 min before the addition of phenylephrine. Each point represents the mean for 4 muscle strips and S.E.M. is shown by vertical bar.

3.4. cGMP contents

As shown in Fig. 4A, the cGMP content was increased to 200% in the 6-h control aorta compared to that in the 0-h control aorta. In the 24-h control aorta, however, there was no additional increase in the cGMP content. In the 6-h interleukin-1β aorta, cGMP content increased to 2670%. In the 12-h interleukin-1β aorta and 24-h interleukin-1β aorta, the increase in cGMP content was smaller than that

in the 6-h interleukin-1β aorta (1030% and 750%, respectively).

As shown in Fig. 4B, addition of 100 μM L-NMMA almost completely inhibited the increase in the cGMP content in the 24-h control aorta and in the 24-h interleukin-1β aorta. Fig. 4B also shows that the increase in cGMP content induced by 1 μM sodium nitroprusside was significantly lower in the 2-h interleukin-1β aorta than in the 24-h control aorta.

Addition of L-NMMA (1 mM) during pretreatment with DMEM did not affect the cGMP content in the 24-h control aorta and in the 24-h interleukin-1β aortas (Fig. 4C). Sodium nitroprusside (1 μM) significantly increased the cGMP content in the 24-h interleukin-1β aorta treated with L-NMMA for 24 h by 1.8-fold compared with that in the 24-h interleukin-1β aorta (Fig. 4C).

3.5. The effect of membrane depolarization

The 24-h control aorta and the 24-h interleukin-1β aorta were stimulated with cumulative addition of KCl. The maximum contraction induced by KCl in the 24-h interleukin-1β aorta (3.2 ± 0.4 mN/mg wet weight, $n = 5$) was not significantly different from that in the 24-h control aorta (2.9 ± 0.6 mN/mg wet weight, $n = 5$, $P > 0.05$). However, the EC_{50} for KCl in the 24-h interleukin-1β aorta (26.1 mM) was significantly higher than that in the 24-h control aorta (15.7 mM, $P < 0.05$) (Fig. 5).

Addition of 25.4 mM KCl and L-NMMA (100 μM) slightly contracted the 24-h interleukin-1β aorta (0.6 ± 0.1 mN/mg wet weight measured 30 min after the addition of KCl and L-NMMA, $n = 5$). In the presence of 25.4 mM KCl and L-NMMA (100 μM), the phenylephrine-induced contraction in the 24-h interleukin-1β aorta was fully restored, increasing the maximum contraction to 103% and decreasing the EC_{50} from 578 to 10.0 nM (Fig. 6A).

Addition of the non-selective K^+ channel inhibitor, tetraethylammonium (30 mM), caused contraction in the 24-h control aorta (2.9 ± 0.6 mN/mg wet weight), but not in the 24-h interleukin-1β aorta. In the presence of tetraethylammonium (30 mM), however, the phenylephrine-induced contraction was enhanced in the 24-h interleukin-1β aorta, increasing the maximum contraction from 15% to 46% of the 24-h control aorta, as shown in Fig. 6B.

4. Discussion

In the first series of experiments, we found that treatment of the muscle with DMEM without interleukin-1β for 6 h increased the cGMP content and inhibited the phenylephrine-induced contraction. Longer treatment with DMEM (24 h) did not cause additional inhibition. Because L-NMMA prevented the effects of DMEM to inhibit contraction and to increase cGMP, it is possible that DMEM treatment activated the induction of NO synthase. Because DMEM did not contain lipopolysaccharide (see Section 2), it is possible that some components of DMEM/fetal bovine serum activated NO synthase induction.

In the aorta treated with interleukin-1β for 6 h, the contraction was more strongly inhibited than that seen after the treatment with DMEM alone. The inhibition was reversed by L-NMMA, supporting the previous report (Ignarro et al., 1981). We also found that a 6-h treatment with interleukin-1β increased the cGMP content 27-fold. Wang et al. (1994) have reported that constitutive NO synthase in the vascular endothelium is up-regulated in the early phase of sepsis. However, since the vascular endothelium was removed in our experiments, the increased cGMP level is not attributable to the endothelial NO synthase, and thus interleukin-1β may induce NO synthase in smooth muscle cells, increase NO production and inhibit constriction.

Because of the low sensitivity of our method, we could not detect the increase in accumulation of NO after a 6-h treatment with interleukin-1β. After treatment with interleukin-1β for 24 h, we detected an increased NO accumulation in DMEM, suggesting that NO production continued up to 24 h in the presence of interleukin-1β. However, the increase in cGMP content was only 28% of that of the 6-h interleukin-1β aorta. These results suggest that the NO/cGMP system is desensitized after a 24-h interleukin-1β treatment. However, the 24-h interleukin-1β treatment suppressed the contraction more strongly than did the 6-h treatment, suggesting that not only NO but also another mechanism is responsible for the inhibition. Supporting this possibility, we found that addition of L-NMMA during tension measurement only partially reversed the inhibitory effect. Furthermore, a selective inhibitor of inducible NO synthase, aminoguanidine, did not reverse the inhibitory effect. From these results, it was concluded that the production of NO may be only partially responsible for the inhibitory effect of the 24-h interleukin-1β treatment.

Although the inhibitory effect of interleukin-1β was significantly restored by methylene blue, the recovery was partial. This result suggests that interleukin-1β has a cGMP-independent mechanism for the inhibition of contraction. Beasley and McGuiggin (1994) reported the NO-independent increase in cGMP induced by interleukin-1β in human but not in rat vascular smooth muscle cells. Furthermore, Wu et al. (1994) reported on the cGMP-dependent but partially NO-independent hyporesponsiveness to norepinephrine of rat aorta induced by lipopolysaccharide. Wu et al. (1994) suggested that the production of interleukin-1β by lipopolysaccharide is involved in the NO-independent activation of guanylate cyclase, but there is another possibility that the effect of interleukin-1β is NO-independent. These results suggest that there may be three different mechanisms of vasodilation, NO-dependent relaxation, NO-independent but cGMP-dependent relaxation, and NO-independent and cGMP-independent relaxation by interleukin-1β.

From our results, the production of cGMP after the treatment with interleukin-1β was maximum at 6 h after the start of treatment and was desensitized at 12 and 24 h after the start of treatment. In contrast to our finding, Beasley et al. (1991) reported that the 24-h interleukin-1β treatment increased the cGMP content to a level similar to that of the 6-h treatment in vascular smooth muscle cells from rat aorta. Recently, however, Papapetropoulos et al. (1996) reported that the interleukin-1β-induced increase of cGMP in rat aorta cells was time-dependent, reaching a peak at 6 h followed by a gradual decrease up to 24 h. Since Beasley et al. (1991) used cells between passages 5 and 18, whereas Papapetropoulos et al. (1996) used cells between passages 1 and 5, these discrepancies may be due not only to differences between cultured cells and freshly isolated cells but also to the different number of passages.

A donor of NO, sodium nitroprusside, increased the cGMP content and inhibited contraction in the control aorta, as reported previously (Shultz et al., 1977; Janis and Diamond, 1979). In the 24-h interleukin-1β aorta, the sodium nitroprusside-induced increase in cGMP content was smaller and the inhibitory effect of sodium nitroprusside was weaker. These results suggest that in the tissue treated with interleukin-1β for 24 h, not only NO synthase but also guanylate cyclase is desensitized. In macrophages, Griscavage et al. (1993) reported that inducible NO synthase is inhibited by NO, possibly because of binding of NO to the heme protein in NO synthase. Since guanylate cyclase contains heme protein, this enzyme may be activated initially and then desensitized by NO. Since a membrane-permeable analogue of cGMP, 8-bromo-cGMP, inhibited contraction in the 24-h interleukin-1β aorta, mechanisms subsequent to the formation of cGMP seemed to be intact. Tsuchida et al. (1994) and Fullerton et al. (1995) have reported that incubation with lipopolysaccharide for 24 h attenuates the sodium nitroprusside-induced relaxation in vascular smooth muscle. Since lipopolysaccharide stimulates the production of interleukin-1β, their finding may also be explained by the desensitization of guanylate cyclase. Furthermore, De Kimpe et al. (1994) reported that guanylate cyclase in bovine mesenteric artery is desensitized by incubation with interferon-γ for 20 h. They also showed that L-NMMA in medium inhibited the desensitization of guanylate cyclase. Recently, Papapetropoulos et al. (1996) reported that, in rat aorta cells, the sodium nitroprusside-induced increase of cGMP was down-regulated by endotoxin or interleukin-1β. Since the protein level of α_1 guanylate cyclase was not altered by endotoxin or interleukin-1β, they suggested that the activity of guanylate cyclase was reduced. These results together with ours suggest that interleukin-1β or lipopolysaccharide down-regulates cGMP production.

Addition of L-NMMA during the 24-h interleukin-1β treatment significantly augmented the inhibitory effect of interleukin-1β. The results for measurement of NO suggested that 1 mM L-NMMA inhibited the synthesis of NO during the 24-h interleukin-1β treatment. It was also found that sodium nitroprusside increased cGMP in the 24-h interleukin-1β aorta treated with L-NMMA but not in the 24-h interleukin-1β aorta without L-NMMA. These results suggest that L-NMMA prevented desensitization of the NO/cGMP system. Because L-NMMA inhibits NO production but not the induction of NO synthase, guanylate cyclase may not be desensitized by NO in the presence of L-NMMA. During measurements of contraction (in the absence of L-NMMA), NO synthase produced a large amount of NO and inhibited the contraction. This possibility was supported by the fact that addition of L-NMMA during tension measurements reversed the inhibitory effect.

Since the maximum force induced by cumulative addition of KCl was not affected by a 24-h treatment with interleukin-1β, contractile proteins may not be inhibited by the interleukin-1β treatment. In contrast, interleukin-1β treatment increased both the threshold concentration and the EC_{50} of KCl. This result suggests that the 24-h interleukin-1β treatment hyperpolarizes the membrane potential of rat aorta cells. To investigate this suggestion, we did another experiment using an agent which induces depolarization of the membrane potential.

The contraction induced by phenylephrine was restored in the presence of high K^+. This result may indicate that the interleukin-1β treatment hyperpolarizes the membrane potential and inhibits the contraction induced by phenylephrine.

The K^+ channel is important to the generation of the membrane potential. In the normal rat aorta, the non-selective inhibition of K^+ channels by tetraethylammonium causes a large contraction in vascular smooth muscle, which is inhibited by a Ca^{2+} channel blocker (Nishio et al., 1986). This result suggests that tetraethylammonium depolarizes the membrane potential and increases Ca^{2+} influx through the voltage-dependent Ca^{2+} channel. In the present experiment, tetraethylammonium enhanced the phenylephrine-induced contraction in the interleukin-1β aorta. This result suggests that interleukin-1β opens K^+ channels and hyperpolarizes the membrane potential, and that the block of K^+ channel opening repressed the inhibitory effect of interleukin-1β.

Because NO has been shown to directly act on K^+ channels and hyperpolarize the membrane potential (Bolotina et al., 1994), we added an inhibitor of NO synthase, L-NMMA, during the interleukin-1β treatment and also during measurement of contraction to inhibit NO production. However, the inhibitory effect of interleukin-1β was not reversed, suggesting that membrane hyperpolarization is not due to a direct interaction between NO and K^+ channels.

Vasodilation induced by interleukin-1β is suggested to be one of the pathogenetic mechanisms of septic shock. Recently, Wei et al. (1995) reported that mutant mice lacking inducible NO synthase survived challenge with endotoxin for 4 days in spite of a 50% mortality in

wild-type mice, suggesting that the mortality associated with endotoxin is largely due to inducible NO synthase in the mouse. In contrast, the hypotensive effect of lipopolysaccharide was not completely reversed by NO synthase inhibitors (Nava et al., 1991; Evans et al., 1994), suggesting that the pathogenesis of vasodilation during human septic shock is not solely due to NO synthase induction.

In conclusion, our results suggest that the treatment of the rat aorta with interleukin-1β for 6 h induced NO synthase, produced large amounts of NO/cGMP and induced vasodilation, whereas treatment for 24 h desensitized the NO/cGMP system and inhibited the contraction by an additional mechanism, possibly membrane hyperpolarization.

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