

## Interleukin 1 Inhibits Contraction of Vascular Smooth Muscle

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### Abstract

Interleukin 1 has been implicated as a mediator of both systemic and local responses to infection and injury. Since systemic and local vasodilatation are hallmarks of sepsis and infection, we studied the direct effect of IL-1 on vascular contractility. We report here that human recombinant IL-1-beta potently inhibits the response of rat thoracic aorta to vasoconstrictor agents. Exposure of isolated rat aortic rings to IL-1 (20 ng/ml) for 1 h did not affect phenylephrine-induced contractions during the exposure period. However, when rings were retested 150–200 min after initiation of IL-1 exposure, contractions were markedly decreased. The cytokine had a similar effect in rings from which the endothelium was removed. Contractions caused by potassium depolarization also were depressed, indicating the effect of IL-1 is not specific to the alpha-adrenoceptor agonist. The inhibitory effect of IL-1 was concentration-dependent (0.2 to 20 ng/ml), and eliminated by pretreatment with cycloheximide (20 µg/ml). Indomethacin ( $10^{-5}$  M) did not prevent the inhibition caused by IL-1.

These studies identify IL-1 as a potent inhibitor of vascular contraction, via an endothelium-independent mechanism. Studies with inhibitors suggest that the action of IL-1 is independent of prostanoïd synthesis, and may involve synthesis of protein.

### Introduction

The cytokine interleukin 1 (IL-1) has been implicated as an important mediator of several generalized host responses to infection and injury. IL-1 administered systemically produces an acute-phase response including fever and neutrophilia, lymphocyte activation, and increased synthesis of hepatic acute phase proteins (1). Tissue production of IL-1 is also thought to play a role in local inflammatory responses.

Systemic vasodilatation that may lead to shock is a major manifestation of sepsis while local vasodilatation is a hallmark of local infection or inflammation. Since recent observations indicate that blood vessels may be an important target for the actions of IL-1 (1–8), we hypothesized that IL-1 may have

direct effects on vascular contraction. The aim of the present study was to determine the effects of IL-1 on blood vessel contraction in vitro.

Preliminary studies indicated that IL-1 did not relax rat aorta precontracted with the alpha-adrenoceptor agonist, phenylephrine. Since many of the effects of IL-1 on blood vessels require several hours to be expressed, the effect of exposure to IL-1 on subsequent contractile responses was determined. Phenylephrine contractions were profoundly depressed in aortic rings treated with IL-1 when tested 150–200 min after initial exposure to the cytokine.

### Methods

Smooth muscle contractility was studied in rings of thoracic aorta isolated from male Sprague-Dawley rats (300–350 g). The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and killed by exsanguination following injection of sodium heparin (100 U i.v.). The aorta was cleaned of adherent connective tissue and cut into rings 5 mm in length. The endothelium was removed mechanically from some of the rings by inserting small forceps into the lumen and gently rolling the ring on a wet filter paper (9). The rings were suspended from strain gauges so as to measure circumferential isometric force and placed in organ chambers containing 25 ml physiological salt solution (PSS, pH 7.4, of the following mM composition: NaCl 118.3, KCl 4.7, MgSO<sub>4</sub> 0.6, KH<sub>2</sub>PO<sub>4</sub> 1.2, CaCl<sub>2</sub> 2.5, NaHCO<sub>3</sub> 25.0, CaEDTA 0.026, and glucose 11.1). The PSS was maintained at 37°C and gassed with 95% O<sub>2</sub>–5% CO<sub>2</sub>. The rings were stretched stepwise to adjust resting tension to 4 g, and allowed to equilibrate for 60 min. After each concentration-response, the rings were washed at least three times with 25 ml fresh PSS and allowed to equilibrate for at least 30 min.

**Phenylephrine contractions.** The effect of IL-1 on contractions to phenylephrine was determined in intact and endothelium-denuded rings. Following equilibration in the organ chambers, the response of the rings to acetylcholine was tested as an indicator of the presence or absence of endothelium (9). The rings were contracted 1.5–2.0 g with phenylephrine, and the relaxation caused by acetylcholine ( $10^{-7}$  to  $10^{-5}$  M) was determined. Intact rings relaxed  $80\pm 10\%$  in response to acetylcholine ( $10^{-5}$  M); rings denuded of endothelium relaxed only  $22\pm 4\%$ . Removal of the endothelium also increased the sensitivity of the rat aorta to the contractile effect of phenylephrine (Fig. 2), as previously described (10). The enhanced sensitivity is likely due to loss of the tonic release of endothelium-derived relaxing factor (10).

Contractions to phenylephrine were then tested three times in each ring: before, during, and after a 1-h exposure to human recombinant IL-1 (Fig. 1). The second concentration-response was begun 15 min after adding IL-1 to the organ chamber and completed by the end of the 1-h exposure. The rings were then washed with fresh PSS and the final concentration-response determination was performed 150–200 min after the addition of IL-1 to the organ chamber (beginning 90 min after the IL-1 was washed out). Contractions caused by phenylephrine

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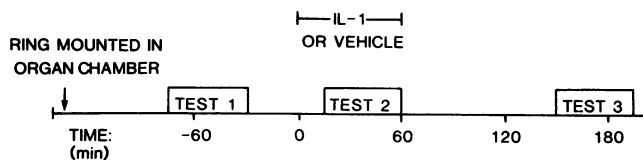
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1. Abbreviations used in this paper: PSS, physiological saline solution.



**Figure 1.** Flow diagram of experimental protocol. "Test" refers to test of contractile response of aortic rings to graded concentrations of phenylephrine. Response to potassium depolarization was studied with the same protocol except that test 2 was omitted.

were tested by increasing the organ chamber concentration by cumulative half-log increments after a steady-state response was reached to each increment. Each phenylephrine response was followed by a short exposure to potassium (120 mM).

**Effect of indomethacin and cycloheximide.** To determine the effect of inhibiting cyclooxygenase, contractions to phenylephrine were tested in rings exposed to indomethacin ( $10^{-5}$  M) during the entire protocol. Control rings were exposed to indomethacin but not IL-1, to verify that the phenylephrine response does not change with time in indomethacin-treated rings.

To test whether the effect of IL-1 was dependent on protein synthesis, phenylephrine responses were also determined in a group of rings exposed to cycloheximide (20  $\mu$ g/ml) throughout the study. This concentration of cycloheximide has been shown to inhibit protein synthesis in rabbit aorta by 85% (assessed by [ $^{35}$ S]methionine incorporation [11]).

**Potassium depolarization.** Contractions caused by depolarization of vascular smooth muscle cells with potassium were tested in intact rings before and 150–200 min after a 1-h exposure to IL-1. The rings were exposed sequentially to 20, 30, 40, and 120 mM potassium PSS, which was prepared by equimolar replacement of sodium.

**Concentration dependence of IL-1 effect.** To determine the relationship between concentration of IL-1 and inhibition of vascular con-

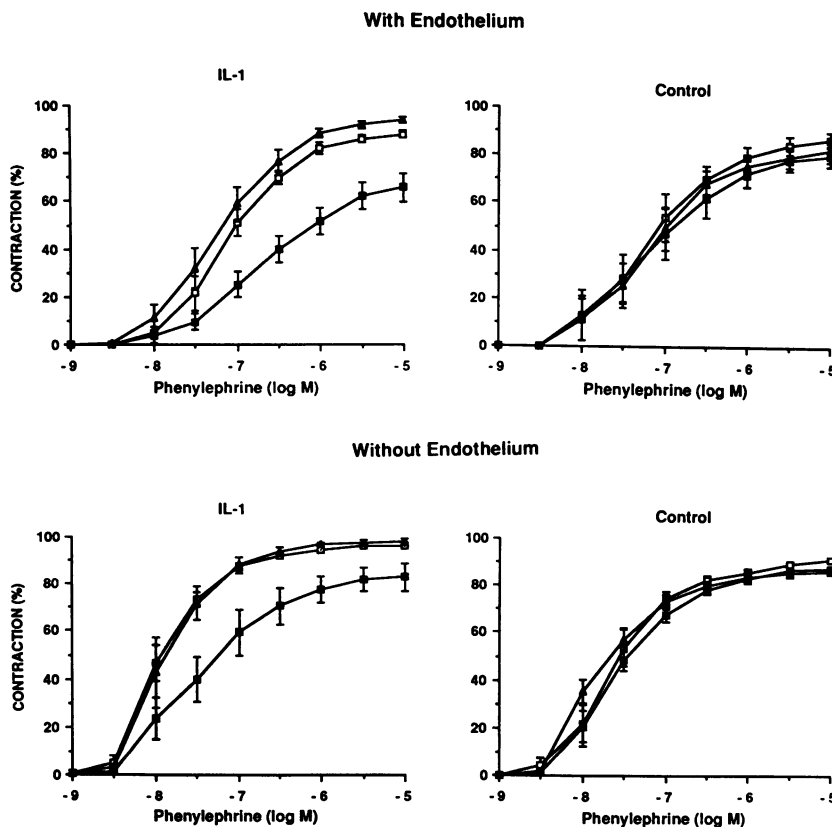
tractility, aortic rings were exposed to 0, 0.2, 2, or 20 ng/ml IL-1 during a 3-h incubation in 0.5 ml PSS in test tubes. The tubes were maintained at 37°C and the PSS was suffused with 95% O<sub>2</sub>/5% CO<sub>2</sub>. The rings were then mounted in the organ chambers, equilibrated for 1 h and the contractions caused by increasing concentrations of phenylephrine were determined.

**Drugs.** Human recombinant interleukin-1-beta was kindly provided by Dr. Alan Shaw of Glaxo Institute of Molecular Biology, Geneva, Switzerland. This preparation of IL-1 produces a half-maximal fever in rabbits at 7.5 ng/kg, and half-maximal stimulation in the murine thymocyte proliferation assay at 0.1 ng/ml. Acetylcholine chloride, 1-phenylephrine hydrochloride, indomethacin, and cycloheximide were obtained from Sigma Chemical Co. (St. Louis, MO).

**Data analysis.** All contractions are expressed as percentages of the first (control) contraction to 120 mM potassium. Data are presented as means  $\pm$  SEM. For all experiments, *n* equals the number of rats from which rings were taken. Significant differences between concentration-response curves were determined by two-way ANOVA for repeated measures. Differences between contractions induced by a single concentration of agonist were determined by paired *t* test. Results were considered statistically significant when *P* < 0.05.

## Results

**Effect of IL-1 on phenylephrine contractions.** Exposure of intact rings to human recombinant IL-1 (20 ng/ml) for 1 h significantly diminished the contractions caused by phenylephrine ( $10^{-9}$  M to  $10^{-5}$  M) in a delayed fashion (Fig. 2, top left). The contractions caused by phenylephrine were not affected significantly during the IL-1 exposure. In contrast, the phenylephrine response tested 150–200 min after initiation of the exposure to IL-1 (i.e., 90 min after washing out the IL-1) was significantly decreased (*n* = 6, *P* < 0.01). At this time, the



**Figure 2.** Effect of IL-1 on phenylephrine-induced contractions in rat thoracic aorta. All contractions are expressed as percentage of the maximal contraction to potassium before treatment with IL-1. (Top left) Phenylephrine responses were studied before exposure to IL-1 ( $\square$ ), during a 1-h exposure to IL-1 ( $\blacktriangle$ ) and 150–200 min after initiation of the IL-1 exposure ( $\blacksquare$ ). Phenylephrine-induced contractions were unaffected during IL-1 exposure but significantly depressed following IL-1 exposure (*n* = 6, *P* < 0.01). (Bottom left) Phenylephrine-induced contractions were also significantly decreased 150–200 min after IL-1 exposure in rings without endothelium (*n* = 5, *P* < 0.02). (Top and bottom right) Control rings studied simultaneously with treated rings (with endothelium *n* = 5, without endothelium *n* = 6), showed no significant changes in response during the duration of the experiments. Symbols refer to corresponding time periods.

contractions caused by phenylephrine ( $10^{-7}$  M) were decreased from  $51\pm6\%$  to  $26\pm6\%$  ( $P < 0.01$ ), or  $\sim 50\%$ . The contractions caused by the maximally effective concentration of phenylephrine ( $10^{-5}$  M) were decreased from  $88\pm2\%$  to  $66\pm6\%$  ( $P < 0.01$ ), representing a 25% inhibition. The contractions of control rings not exposed to IL-1 were unchanged when tested simultaneously with the treated rings (Fig. 2, right).

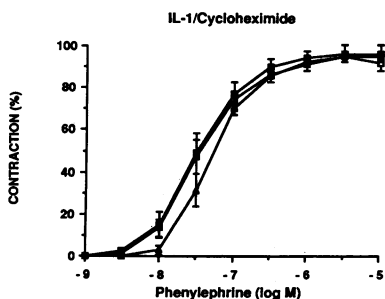
**Influence of the endothelium.** The inhibitory effect of IL-1 was comparable in rings from which the endothelium had been removed to that in intact rings (Fig. 2, bottom left). Phenylephrine-induced contractions were significantly decreased 150–200 min after initiation of the exposure to IL-1 ( $n = 5$ ,  $P < 0.02$ ). Since endothelium-denuded vessels are more responsive to phenylephrine, the depressant effect of IL-1 is best compared at concentrations of phenylephrine that produced contractions of comparable magnitude in intact and denuded control rings. Contractions caused by phenylephrine ( $10^{-8}$  M) were decreased significantly from  $47\pm8\%$  to  $23\pm9\%$  ( $P < 0.05$ ) in endothelium-denuded vessels, which is comparable to the decrease observed in contractions of intact vessels to phenylephrine ( $10^{-7}$  M).

**Cycloheximide.** In the presence of cycloheximide (20  $\mu\text{g/ml}$ ), IL-1 had no significant effect on phenylephrine contractions (Fig. 3).

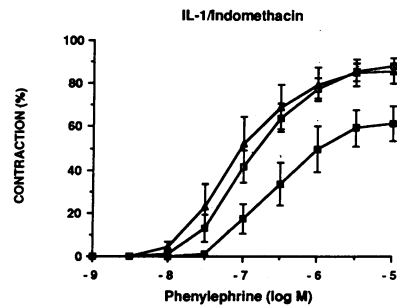
**Indomethacin.** The depression in phenylephrine-induced contractions caused by IL-1 was unaffected by indomethacin ( $10^{-5}$  M; Fig. 4). Contractions were decreased significantly 150–200 min after initiation of exposure to IL-1 ( $n = 5$ ,  $P < 0.01$ ). Contractions caused by phenylephrine ( $10^{-7}$  M) were decreased from  $42\pm8\%$  to  $17\pm7\%$  ( $P < 0.01$ ) following IL-1 exposure, comparable to the effect of IL-1 in rings not exposed to indomethacin. Phenylephrine responses did not change in time-control rings treated with indomethacin but not exposed to IL-1.

**Potassium contractions.** The contractions of rings caused by depolarization with potassium (Fig. 5) also were decreased significantly 150–200 min following a 1-h exposure to IL-1 (20 ng/ml;  $n = 6$ ,  $P < 0.01$ ). The maximal contraction to potassium was significantly decreased by  $12\pm2\%$  ( $P < 0.01$ ). Contractions caused by potassium were unchanged in control rings that were not exposed to IL-1.

**Concentration dependence of IL-1 inhibition.** Exposure of aortic rings to IL-1 for 3 h before mounting in the organ chamber produced a concentration-dependent inhibition of contractions to phenylephrine (Fig. 6). After a 3-h exposure to 0.2, 2.0, or 20 ng/ml IL-1, contractions caused by phenylephrine ( $10^{-7}$  M) were decreased significantly from  $59\pm5\%$  in control rings not exposed to IL-1 to  $42\pm5\%$  ( $P < 0.05$ ),  $31\pm10\%$  ( $P < 0.02$ ), and  $8\pm3\%$  ( $P < 0.001$ ), respectively.



**Figure 3.** Effect of IL-1 on contractions to phenylephrine in aortic rings treated with cycloheximide ( $n = 4$ ). The rings were exposed to cycloheximide (20  $\mu\text{g/ml}$ ) during the entire experiment. Protocol and symbols as in Fig. 2.



**Figure 4.** Effect of IL-1 on contractions induced by phenylephrine in aortic rings exposed to indomethacin ( $n = 5$ ). Indomethacin ( $10^{-5}$ ) was present during the entire experiment. Protocol and symbols are as in Fig. 2. Phenylephrine-induced contractions were significantly decreased 150–200 min after exposure to IL-1 ( $P < 0.01$ ).

## Discussion

These studies demonstrate for the first time that the cytokine IL-1 is a potent inhibitor of vascular smooth muscle contractility. IL-1 diminished the maximal contractions in response to either an  $\alpha$ -adrenoceptor agonist or direct depolarization of the smooth muscle. The inhibitory effect of IL-1 on contractions in blood vessels denuded of endothelium was comparable to the effect in intact vessels. In contrast, the relaxation caused by the endothelium-dependent vasodilator, acetylcholine, was largely eliminated. Thus, IL-1 appears to act directly on vascular smooth muscle cells.

Previous studies have described a wide variety of changes in response to IL-1 in cultured vascular smooth muscle and endothelial cells (1). In cultured endothelial cells, IL-1 induces the synthesis of vasodilators including  $\text{PGE}_2$  and  $\text{PGI}_2$  (2) and platelet activating factor (3), induces shape changes (4), and elicits a series of responses that promote blood coagulation (5) and increase adhesion of leukocytes (6). Vascular smooth muscle cells in culture also produce  $\text{PGE}_2$  and  $\text{PGI}_2$  (2) and proliferate (7) upon exposure to IL-1.

Depression of vascular contractility was not apparent during the 1-h exposure to IL-1, but was apparent 150–200 min after initial exposure to the cytokine. The delayed action of IL-1 suggests a mechanism involving protein synthesis. This hypothesis is supported by the fact that IL-1 had no effect on contractility in aortic rings treated with cycloheximide. Stimulation of prostacyclin (2), procoagulant activity (5), and platelet activating factor (3) in cultured endothelial cells by IL-1 requires several hours to develop, and the induction of platelet activating factor by IL-1 is blocked by cycloheximide (3). As the action of IL-1 on vascular contractility appears to require synthesis of protein, the effect of IL-1 may represent a novel mechanism for prolonged alterations of vascular responsiveness.

Recent evidence indicates that IL-1 can induce a shock-like state in rabbits (8). The hypotension induced by IL-1 is a result of decreased systemic vascular resistance, whereas cardiac output is increased. Inhibition of vascular smooth muscle contractility by a mechanism similar to the one described here could contribute to the hypotension caused by systemic administration of IL-1. However, there are two differences between the *in vivo* hypotensive effect of IL-1 and the inhibitory effect of IL-1 on rat aorta *in vitro*. First, blood pressure falls within 10 min of intravenous injection and reaches its nadir within 40 min, whereas the effect of IL-1 on aortic rings is not

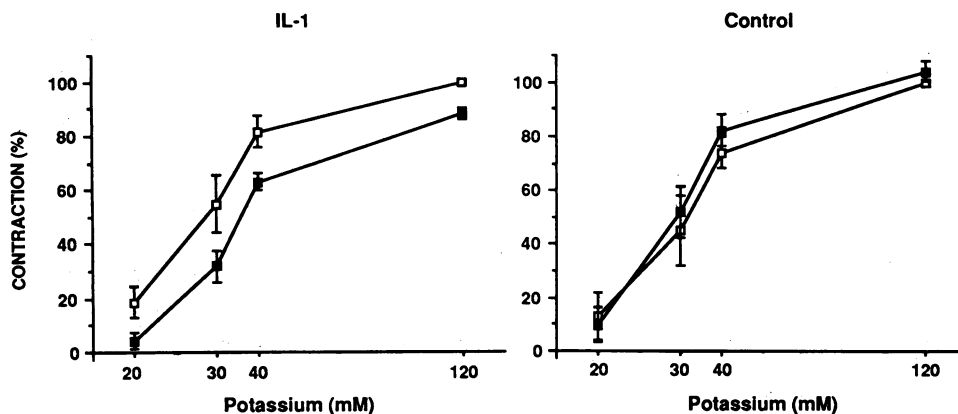


Figure 5. Effect of IL-1 on contractions caused by high potassium PSS. K responses were studied before exposure to IL-1 (—□—) and 150–200 min after the beginning of a 1-h exposure to the cytokine (—■—). Contractions were significantly diminished 150–200 min following exposure to IL-1 (left,  $n = 6$ ,  $P < 0.01$ ), and unchanged in time control rings that were not exposed to IL-1 (right,  $n = 4$ ).

apparent during the first hour. Second, hypotension caused by IL-1 in vivo could be reversed or prevented with a single injection of ibuprofen, suggesting an important role for prostanooids. In contrast, it is unlikely that the inhibitory effect of IL-1 on rat aorta is mediated by prostanooids since the effect was not blocked by indomethacin. Thus, the mechanism described here is not likely to be operative in the induction of the early stages of shock by IL-1 in rabbits. Conceivably the delayed reduction in contractility we report here may play a role in later phases of shock mediated by IL-1.

The inhibitory effect of IL-1 on vascular contraction was concentration-dependent over a range of 0.2 to 20 ng/ml. The concentrations of IL-1 are similar to the concentrations that induce other well established effects of IL-1 in vitro, such as induction of PGE<sub>2</sub> production in human fibroblasts (12). Although it is not known whether concentrations of IL-1 used for experiments in vitro correspond to tissue concentrations of IL-1 in vivo, endogenous IL-1 is thought to play an important role in inflammatory processes (1). Plasma concentrations of 0.2–0.4 ng/ml have been reported in normal human subjects (13), and plasma concentrations increase to 1.5 ng/ml during severe infection in children (14). Our studies raise the possibility that endogenous IL-1 plays a role in modulating vascular

reactivity under physiological or pathophysiological conditions. The primary sources of endogenous IL-1 are thought to be circulating monocytes and tissue macrophages. Since IL-1 is produced locally, tissue levels of IL-1 may exceed plasma levels. Recent evidence indicates that both vascular smooth muscle (15) and vascular endothelial (16) cells in culture also produce IL-1. Vascular contractility may be affected by IL-1 produced locally by resident macrophages or by vascular cells, as well as by circulating IL-1.

IL-1-induced depression of vascular contractility may play a role in pathological conditions such as the decreased vascular resistance associated with bacterial sepsis. In addition, IL-1 released locally by vascular cells or macrophages may play an important role in local hyperemia associated with sites of inflammation. Further study of IL-1-induced modulation of vascular contractility may provide insight into a wide spectrum of vascular diseases.

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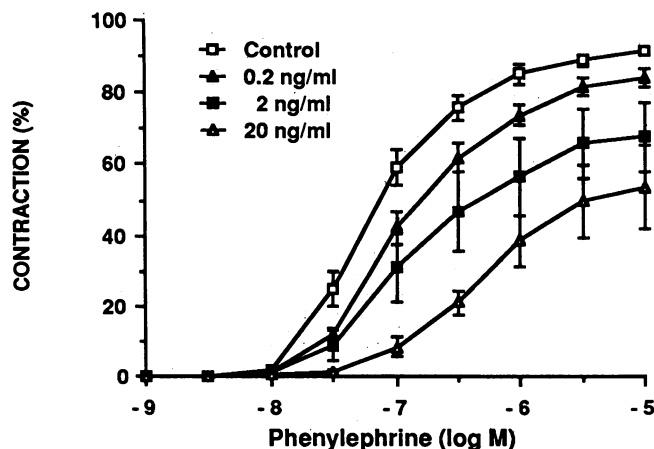


Figure 6. Relationship between concentration and the inhibitory effect of IL-1 on phenylephrine contractions. Rings were exposed to either 0, 0.2, 2.0, or 20 ng/ml IL-1 for 3 h in vitro, mounted in the organ chambers, equilibrated at resting tension for 1 h and administered phenylephrine ( $n = 5$  for each concentration of IL-1).

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