Interleukin-1β alters actin expression and inhibits contraction of rat thoracic aorta

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Trinkle, Laura A., Debbie Beasley, and Robert S. Moreland. Interleukin-1β alters actin expression and inhibits contraction of rat thoracic aorta. Am. J. Physiol. 262 (Cell Physiol. 31): C828–C833, 1992.—Previous studies have indicated that interleukin-1β (IL-1) inhibits contraction of rat aortas by activating nitric oxide production in vascular smooth muscle cells, with subsequent increases in guanosine 3',5'-cyclic monophosphate (cGMP). This study determined if the effect of IL-1 involves the primary regulatory event in smooth muscle activation, myosin light chain (MLC) phosphorylation. This study also examined whether IL-1 affects contractile protein content. IL-1 (20 ng/ml) significantly decreased stress in response to 0.1 μM phenylephrine with a concomitant decrease in MLC phosphorylation. Incubation with IL-1 for 3 h or longer decreased α-smooth muscle actin and increased γ-actin isoform, with no change in β-nonmuscle actin or myosin isozyme content. These results suggest that IL-1 inhibition of a vascular smooth muscle contraction may be due to a decrease in activator calcium, which may account for the resultant decrease in MLC phosphorylation. These results also indicate that IL-1 significantly affects contractile protein content, enhancing γ-actin isoforms and decreasing the vascular smooth muscle specific α-isoactin.

Vascular smooth muscle; phosphorylation; myosin light chain; myosin; isoforms; phenylephrine

INTERLEUKIN-1β (IL-1) is a pleiotropic cytokine that is produced primarily by monocytes and macrophages, as well as by many nonimmune cell types when activated with bacterial lipopolysaccharide (LPS; for reviews see Refs. 7, 16). Systemic effects of circulating IL-1 are thought to mediate many of the acute phase responses to infection and LPS administration including fever, whereas IL-1 produced within tissues contributes to local inflammatory responses (7). Recent evidence indicates that direct actions of IL-1 on vascular smooth muscle cells are also likely to contribute to systemic and local vasodilation, which is associated with sepsis and local infection; an IL-1 receptor antagonist prevents LPS-induced hypotension (28). IL-1 causes hypotension when infused in vivo (19), and IL-1 has vasodilatory actions on blood vessels in vitro (15).

Vasodilatory actions of IL-1 are likely to involve multiple mechanisms. IL-1 induces the synthesis of vasodilatory prostanoids in cultured human vascular smooth muscle cells (22), causes prostanoïd-dependent hypotension in rabbits in vivo (19), and inhibits contraction of rabbit aortic rings by a prostanoïd-dependent mechanism (15). In rat aortic rings, IL-1 inhibits contraction by activating nitric oxide production in vascular smooth muscle cells, with subsequent activation of soluble guanylate cyclase and increases in guanosine 3',5'-cyclic monophosphate (cGMP) (1–3). The effect of IL-1 on vascular contraction at the level of the contractile proteins, however, has not been investigated. Because many of the actions of IL-1 are dependent on protein synthesis (7), we proposed that IL-1 may also affect the contractile state of vascular smooth muscle by altering the expression and function of contractile proteins.

The primary regulatory event in the initiation of agonist-induced contraction is the phosphorylation of the 20-kDa myosin light chain (MLC) catalyzed by the calcium- and calmodulin-dependent MLC kinase (for review, see Ref. 9). The present study compared agonist-induced MLC phosphorylation in control and IL-1-treated rat aortas to assess whether IL-1 inhibits agonist-induced contraction at a step proximal or distal to the MLC kinase (i.e., at the level of increases in cytosolic free calcium or calcium-dependent MLC phosphorylation as compared with alterations in the contractile filaments’ response to MLC phosphorylation). We also addressed the question of whether IL-1 affects contractile protein content of vascular smooth muscle by quantifying the expression of actin and myosin isoforms in control and IL-1-treated rat aortas.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250–275 g) were killed by CO2 overdose, and the thoracic aorta was removed and cleaned of loose fat and connective tissue. Circumferential strips, ~2 mm in width, were incubated for 3 h in either 1 ml of physiological salt solution (PSS) of the following composition (in mM): 140 NaCl, 4.7 KCl, 1.2 MgSO4, 1.6 CaCl2, 1.2 NaH2PO4, 2.2-(N-morpholinopropanesulfonic acid (MOPS; pH 7.4 at 37°C), 5.6 D-glucose, and 0.02 Na2-EDTA or in 1 ml of PSS containing 20 ng/ml human recombinant IL-1 (kindly provided by Dr. Charles Dinarello or obtained from Cistron Biotechnology), then examined for IL-1-induced alterations in either contractile function or protein composition. The concentration of IL-1 and length of incubation were similar to that used in previous studies (1, 2). The aortic strips were not oxygenated during this incubation. Control experiments with aortic strips mounted for isometric force recording immediately after dissection produced similar levels of agonist-induced force as those incubated in PSS for 3 h.

Determination of contractile function was performed by measurement of isometric force in response to phenylephrine. The vascular strips were mounted in water-jacketed muscle chambers by means of two plastic clips, one of which was attached to a micrometer for control of muscle length and the other attached to a Grass FT.03 force transducer and a model 7 Grass polygraph. The strips were bathed in PSS, aerated with 100% O2, and maintained at 37°C. The strips were then stretched to produce a force of 0.5 g and allowed to stress-relax for at least 45 min. An initial contraction with 0.1 μM phenylephrine determined viability of the vascular preparation, which was then rinsed with PSS and allowed to fully relax. Repeated stretches over a 90-min period were imposed to establish a stable preload of 0.5 g. The strips were then contracted with
0.1 μM phenylephrine until a reproducible contraction was elicited. All forces were normalized to tissue cross-sectional area and expressed as stress (in N/m²).

The temporal relationship between force and MLC phosphorylation was determined during a 30-min contraction in response to 0.1 μM phenylephrine. This agonist, phenylephrine, and concentration were chosen because previous studies (2) indicated that 20 ng/ml IL-1 significantly inhibits contractile responses to phenylephrine (10 nM–10 μM) and 0.1 μM phenylephrine is the concentration which produces a half-maximal response in control aortic strips and demonstrates the greatest depression following IL-1 treatment. Eight strips from a single aorta were contracted with 0.1 μM phenylephrine and rapidly frozen at various times during the contraction in a dry ice-acetone slurry containing 6% trichloroacetic acid. The frozen strips were slowly thawed and homogenized in a solution containing 20 mM dithiothreitol, 10% glycerol, and 1% sodium dodecyl sulfate (SDS). The homogenized strips were subjected to two-dimensional electrophoresis as previously described (18). The electrophoresed proteins were visualized by Coomassie Blue staining, followed by exhaustive destaining until the gel cleared, and then silver stained using a Bio-Rad silver staining kit. The initial Coomassie Blue staining has been previously reported to significantly enhance the sensitivity and linearity of silver staining (5). Quantitation of MLC phosphorylation levels was performed by scanning densitometry using an LKB model 2202 laser densitometer interfaced to an LKB 2220 recorder-integrator. Phosphorylation values were calculated by integration of the electrophoretic spot corresponding to the phosphorylated MLC as a percent of the total integration of both the phosphorylated and unphosphorylated MLC. The satellite spots were rarely seen and were not taken into account in the calculations.

Actin isoform quantitation was performed on strips homogenized as above. The homogenized strips were subjected to two-dimensional electrophoresis as described by Fatigati and Murphy (8) and visualized by Coomassie Blue staining. Quantitation of the actin isoforms was performed by image analysis with a CCD camera and a video digitizing board interfaced to an IBM-XT microcomputer using Jandel Scientific JAVA software. The individual isoactins were identified by comparison with published data (8). Each actin isoform was expressed as a percent of the total actin content.

Two myosin heavy chain isoforms, termed SM1 and SM2 in order of increasing electrophoretic mobility by Rovner et al. (23), were quantitated by one-dimensional SDS gel electrophoresis. The aortic strips were homogenized in 25 mM NaHPO4, 1% β-mercaptoethanol, and 1% SDS as described by Seidol and Murphy (24). The homogenized strips were subjected to SDS electrophoresis with an acrylamide concentration of 4%. Because of the low acrylamide concentration, the gels were supported with Gelbund PAG film (FMC Bioproducts). Quantitation of the Coomassie Blue-stained gels was performed using a Helena Laboratories Quick Scan R&D densitometer interfaced to an Apple Macintosh plus computer using Hoefer Scientific Instruments integration software (GS-370). Each myosin heavy chain isoform was expressed as a percent of the total myosin content.

The ratio of total actin to total myosin as well as the ratios of either total actin or total myosin to total aortic protein were determined by one-dimensional SDS gel electrophoresis. Aortic tissues homogenized as described for the determination of MLC phosphorylation levels were subjected to SDS gel electrophoresis using an acrylamide concentration of 7.5%. Quantitation of total electrophoresed protein, actin, or myosin was performed as described for the determination of myosin isoforms.

Significant differences between means of all values were determined using the Student’s t test for unpaired data. A P value < 0.05 was taken as significant.

RESULTS

Stress and MLC phosphorylation. The temporal relationship of stress and MLC phosphorylation during a 30-min exposure of the aortic strips to 0.1 μM phenylephrine was determined in both control and IL-1-treated tissues. The results of these studies are shown in Figs. 1 and 2. Stress developed to 1.5 ± 0.2 × 10⁶ N/m² and was maintained in the control aortic strips (Fig. 1) in response to 0.1 μM phenylephrine stimulation. This monotonous increase in stress was accompanied by a transient increase in MLC phosphorylation levels (peak value, 0.36 ± 0.05 mol P/mol MLC) typical of vascular smooth muscle (18).

Stress also developed in response to phenylephrine stimulation in the IL-1-incubated aortic strips, but in contrast to the response in the control tissues, stress developed to significantly lower levels (1.0 ± 0.1 × 10⁶ N/m²; P < 0.05 as compared with control) and was poorly

![Fig. 1](image-url) Time-dependent profile of stress and myosin light chain (MLC) phosphorylation in response to 0.1 μM phenylephrine in control rat thoracic aorta. Phenylephrine caused rapid development and maintenance of stress during a transient increase in MLC phosphorylation. Values shown are means ± SE (n = 3–12 determinations).

![Fig. 2](image-url) Time-dependent profile of stress and MLC phosphorylation in response to 0.1 μM phenylephrine in interleukin-1 (IL-1)-incubated rat thoracic aorta. Phenylephrine-induced contractions were not maintained, and the characteristic transient MLC phosphorylation profile exhibited a biphasic profile. There were no significant effects on either the basal or steady-state levels of MLC phosphorylation following incubation in IL-1. Values shown are means ± SE (n = 9 determinations).
maintained during the stimulation period (Fig. 2). More striking is the time-dependent profile of MLC phosphorylation levels in the IL-1-incubated tissues. In contrast to the single transient in MLC phosphorylation (Fig. 1), following IL-1 incubation MLC phosphorylation levels exhibited a biphaseic increase. MLC phosphorylation increased to an initial peak at 0.5 min (0.27 ± 0.03 mol P/mol MLC; \( P < 0.05 \) as compared with control), which by 1 min was significantly decreased (0.14 ± 0.02 mol P/mol MLC). A second peak in MLC phosphorylation occurred at 2 min (0.22 ± 0.04 mol P/mol MLC) followed by a slow decline to suprabasal levels.

To examine how IL-1 affected the relationship between stress and MLC phosphorylation, the stress data in Fig. 1 were normalized as a percent of the maximal response. The percent decrease in maximal developed stress following IL-1 treatment as compared with control was 0.67. This inhibition factor was used to normalize the stress data following IL-1 incubation shown in Fig. 2. This was performed by dividing all values of stress obtained following IL-1 incubation by 0.67, thus effectively abolishing the difference in developed stress. The exact same procedure, division by 0.67, was used to normalize the MLC phosphorylation data following IL-1 incubation shown in Fig. 2. The same inhibition factor based on the decrease in stress was used in the normalization of MLC phosphorylation so as not to obscure any differential changes in stress and MLC phosphorylation that may have occurred following IL-1 incubation. MLC phosphorylation levels determined in control strips were not altered from that shown in Fig. 1. These normalized results are shown in Fig. 3, which demonstrates that although in absolute magnitude, both MLC phosphorylation and stress are reduced by IL-1 exposure, the relationship between these parameters during the initial stress development phase of the contraction is unchanged. Figure 3 also clearly shows that although the normalized MLC phosphorylation values are similar, stress is not maintained following IL-1 incubation, whereas it is in the control aortic strips.

**Actin isoforms.** Three isoforms of actin are present in smooth muscle: α-smooth muscle actin, β-nonmuscle actin, and γ-muscle and nonmuscle actin. Figure 4 shows the relative proportions of each of these actin isoforms in control and IL-1-treated rat aortic tissue. As can be seen from Fig. 4, incubation with IL-1 significantly altered the isoform profile. Incubation with IL-1 resulted in a significant increase in the γ-isoform of actin (17.0 ± 3.1% IL-1 vs. 7.7 ± 1.7% control; \( P < 0.05 \)), a significant decrease in the α-smooth muscle actin (64.5 ± 2.2% IL-1 vs. 72.0 ± 2.1% control; \( P < 0.05 \)), and no change in the β-nonmuscle actin (18.3 ± 1.5% IL-1 vs. 20.3 ± 1.4% control; \( P > 0.05 \)). The ratio of total actin to either myosin or total protein was examined to determine whether the apparent shift in fractional isoactin content was the result of a relative increase in total actin or an absolute change in the specific actin isoform. These data are shown in Table 1 and demonstrate that total actin content did not increase following IL-1 exposure, suggesting an absolute change in both α- and γ-actin isoforms.

To determine if the IL-1-induced decrease in stress development is associated with the change in actin isoform profile shown in Fig. 4, we examined the time dependence of both parameters. The aortic strips were incubated in PSS or PSS containing 20 ng/ml IL-1 for

![Fig. 3. Normalized stress and MLC phosphorylation profiles in control and IL-1-incubated rat thoracic aorta. Levels of stress (m) from control strips were normalized as a percent of their own maximum. Levels of MLC phosphorylation (c) from control strips are actual data. Magnitude of inhibition of stress produced by IL-1 was used to normalize levels of stress (m) from the IL-1-incubated strips. Magnitude of inhibition of stress produced by IL-1 was also used to normalize levels of MLC phosphorylation (c) from the IL-1-incubated strips. See text for further details. The decrease in MLC phosphorylation at 1 min of incubation in the IL-1-incubated strips was omitted for clarity, as were the SE. IL-1 incubation did not affect the relationship between stress development and MLC phosphorylation, although the relationship between stress maintenance and MLC phosphorylation was significantly altered. *Significantly different from control, \( P < 0.05 \).](image)

![Fig. 4. Actin isoform profile for both control and IL-1-incubated strips. Each actin isoform is shown as a percent of the total actin. After IL-1 incubation there is a significant increase in γ-isoform of actin (\( P < 0.05 \)), a significant decrease in α-smooth muscle actin (\( P < 0.05 \)), and no change in the β-nonmuscle actin. Values shown are means ± SE (\( n = 11 \) determinations); *Significantly different from control.](image)

| Table 1. Effect of IL-1 on rat aortic contractile protein content |
|-------------------|--------|--------|
| Ratio             | Control| IL-1   |
| Actin/myosin      | 4.29±0.39 | 4.32±0.27 |
| Actin/total protein| 27.12±0.87 | 29.07±0.94 |
| Myosin/total protein| 5.81±0.23 | 5.66±0.44 |
| SM1/SM2           | 1.19±0.05 | 1.11±0.05 |

Values are means ± SE; \( n \), number of determinations. IL-1, interleukin-1.
1, 2, 3, and 5 h. At the end of the incubation, stress development in response to 0.1 μM phenylephrine of each strip was determined. The strips were then homogenized and subjected to two-dimensional electrophoresis for quantitation of actin isoforms. The results of these experiments are shown in Table 2. These results demonstrate that the IL-1 induced decrease in stress development precedes the alteration in actin isoform profile.

Myosin isoforms. The regulatory proteins of smooth muscle are associated with the thick filaments (for review, see Ref. 9). Therefore, because IL-1 exposure significantly decreases contractile activity, the possibility of changes in myosin heavy chain content and/or isoform expression was examined. The two myosin isoforms, SM1 and SM2, were quantitated in control strips of rat aorta and strips of aorta following exposure to IL-1. The results of these determinations are shown in Table 1. As shown in Table 1, IL-1 had no significant effect on either the relative proportion of the two myosin isoforms or on the total myosin content relative to total tissue protein, suggesting that the cellular changes induced by IL-1 may be limited strictly to alterations in the thin filament.

**DISCUSSION**

Our results indicate that IL-1 inhibits agonist-induced contraction in rat aortic smooth muscle by inhibiting phosphorylation of the 20-kDa MLC. MLC phosphorylation was significantly decreased in IL-1-treated tissues as compared with control tissues over the majority of a 30-min contraction in response to phenylephrine. It is well recognized that an increase in cellular Ca²⁺ initiates smooth muscle contraction. It is also widely believed that the initiating step for force development is the Ca²⁺- and calmodulin-dependent activation of the MLC kinase and resultant MLC phosphorylation. In almost all vascular smooth muscle tissues examined, a strong correlation exists between cell Ca²⁺ and the level of MLC phosphorylation during agonist stimulation. The results of this study would therefore suggest that IL-1 treatment decreases the agonist-stimulated increase in cellular Ca²⁺. However, we cannot rule out the equally plausible explanations that IL-1 treatment decreases the activity of MLC kinase (26) or increases the activity of a MLC phosphatase (10) in response to a similar increase in Ca²⁺.

Studies by Beasley and co-workers (1–3) indicate that IL-1 inhibits contraction of rat aortas by inhibiting nitric oxide production in vascular smooth muscle cells, with subsequent increases in cGMP. IL-1-induced inhibition of contraction of rat aortae is independent of prostanoids (2), independent of the endothelium (2), and dependent on protein synthesis (2). Subsequent studies documented that IL-1 increases cGMP in endothelium-denuded rat aortas and that this increase in cGMP and concomitant inhibition of contraction is completely reversed by inhibitors of soluble guanylyl cyclase and by hemoglobin which binds nitric oxide (1). These studies suggested that IL-1 inhibits contraction of this tissue by inducing a nonendothelial source of nitric oxide, with subsequent increases in cGMP. Further studies documented that IL-1 induces nitric oxide production in rat aortic smooth muscle cells (3), supporting the hypothesis that the source of nitric oxide in endothelium-denuded rat aortas is smooth muscle cells. Whereas endothelium-dependent vasodilators induce a rapid, transient, and calcium-dependent activation of nitric oxide synthase in endothelial cells (20), IL-1-induced activation of nitric oxide synthase in vascular smooth muscle cells is dependent on protein synthesis and is prolonged (3); increases in cGMP occur after several hours of exposure and are maintained for many hours. Because cGMP has been shown to decrease cellular Ca²⁺ (for review, see Ref. 14), the results of the present study are most compatible with the hypothesis that prolonged activation of cGMP production in response to IL-1 treatment reduces activator [Ca²⁺], resulting in a decrease in MLC phosphorylation levels and therefore stress development.

An intriguing finding in this study is the IL-1-induced change in the time-dependent profile of MLC phosphorylation from a monophasic to a biphasic transient. The significant decrease in MLC phosphorylation at 1 min of stimulation is not due to experimental variability but occurred in every stimulated set of aortic strips following IL-1 treatment. In contrast, a biphasic response in MLC phosphorylation was never seen in control aortic strips. The protocols used in this study to measure MLC phosphorylation quantitate total phosphorylation and do not determine the residue phosphorylated. It is known that MLC kinase phosphorylates the serine-19 residue (reviewed in Ref. 9), and in addition, it is known that protein kinase C phosphatolyses alternate residues on the MLC, the functions of which are controversial. Precise peptide mapping is necessary to determine which residues are phosphorylated. However, it is interesting to speculate that IL-1 may alter the

Table 2. Time dependence of the IL-1-induced alterations in stress development and actin isoform profile

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Stress</th>
<th>α-Actin</th>
<th>β-Actin</th>
<th>γ-Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>IL-1</td>
<td>Control</td>
<td>IL-1</td>
</tr>
<tr>
<td>1</td>
<td>1.8±0.2 (2)</td>
<td>1.6±0.2 (2)</td>
<td>74.6±5.6</td>
<td>73.8±4.9</td>
</tr>
<tr>
<td>2</td>
<td>1.6±0.2 (2)</td>
<td>1.0±0.2 (2)*</td>
<td>76.2±6.1</td>
<td>74.1±3.0</td>
</tr>
<tr>
<td>3</td>
<td>1.5±0.2 (2)</td>
<td>1.0±0.2 (2)*</td>
<td>72.0±2.1</td>
<td>64.5±2.2</td>
</tr>
<tr>
<td>5</td>
<td>1.4±0.2 (2)</td>
<td>1.0±0.2 (2)*</td>
<td>73.6±3.8</td>
<td>62.6±3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE for 2–5 determinations at 1, 2, 5 and 11 determinations at 3 h. Rat aortic strips were incubated in 20 ng/ml IL-1 for 1, 2, 3, or 5 h and then mounted to allow for isometric force recording. Values shown for stress are the response of the strips to 0.1 μM phenylephrine and are in units of 10⁶ N/m². After the determinations of stress, the strips were homogenized and subjected to 2-dimensional electrophoresis for quantitation of actin isoforms, expressed as percent of total actin content. Although 2 h of exposure to IL-1 was sufficient to significantly depress stress development, significant changes in actin isoform content were not noted until at least 3 h of exposure. * P < 0.05 as compared with control.
functional role of protein kinase C in a vascular smooth muscle contraction.

In addition to the role of Ca\textsuperscript{2+} in the MLC phosphorylation-dependent development of stress, a second role for Ca\textsuperscript{2+} has been postulated for the maintenance of stress. Dillon et al. (6) demonstrated that with continued smooth muscle stimulation, isometric shortening velocity (an estimate of cross-bridge cycling rate) and MLC phosphorylation fall to suprabasal levels while stress is maintained at high levels. This state of maintained stress without proportional MLC phosphorylation, termed the latch state, has been shown to be Ca\textsuperscript{2+} dependent (17, 18). The results of this study (Fig. 1 as compared with Fig. 2) demonstrate that stress is not maintained if the aortas are previously exposed to IL-1. An interesting aspect to this result is the relationship between MLC phosphorylation and stress maintenance following IL-1 treatment (Fig. 3). Although no apparent change is seen in the dependence of stress on MLC phosphorylation during the development phase (0-10 min of stimulation), a distinct separation of stress and MLC phosphorylation in the IL-1-treated strips is seen in the maintenance phase (10-30 min of stimulation). The profile of this contraction is similar to that of KCl-depolarized swine carotid medial strips in the presence of calcium channel blockade with dihydropyridines (18), where stress maintenance is significantly inhibited without any alteration in the levels of MLC phosphorylation. This suggests that either insufficient Ca\textsuperscript{2+} is available to maintain stress or there is an IL-1-induced change in the function and/or content of the contractile protein unit(s) responsible for the maintenance of stress. This also clearly demonstrates that the maintenance of stress does not directly depend on the level of MLC phosphorylation.

A striking finding of this study was the rapid change in actin isoforms occurring after exposure to IL-1. α-Smooth muscle actin was decreased while the γ-isoactin was increased. β-Nonmuscle isoactin was unchanged by IL-1 exposure. Of specific interest is the fact that these changes in actin profile occurred during only a 3- to 5-h period. Although we have no direct information as to whether the increase in the γ-isoactin is due to an increase in the nonmuscle form, the smooth muscle form, or both, we speculate that the increase may be a specific increase in the nonmuscle form. This is based on previous studies demonstrating that in rat aorta, γ-smooth muscle actin comprises only 6% of the total actin content (27) and the expectation that a mitogen such as IL-1 would increase the expression of proteins involved in the growth and secretory responses of the cell, i.e., γ-nonmuscle actin. Our results presented in this study do not specifically address this issue. Thus IL-1 produced cellular changes that resulted in the apparently specific degradation of the α-smooth muscle isoactin and the specific synthesis of γ-isoactin. This demonstrates that although not directly related, IL-1 has dramatic effects on both vascular smooth muscle structure and function.

In contrast to the rapid changes in actin isoforms, no significant change in myosin heavy chain isoforms was demonstrated. Differences in smooth muscle myosin heavy chain may be expected in response to a mitogen such as IL-1, since previous studies have shown that vascular smooth muscle cells undergo differential expression of smooth muscle- and nonmuscle-specific isoforms of the myosin heavy chain at different stages when grown in culture (23). The expression of the nonmuscle form of myosin appears to correlate with changes in their growth state, primarily as the smooth muscle cell loses its contractile properties and becomes more secretory in nature (4, 11). We did not find any significant change in the relative amounts of the two myosin isoforms, SM1 and SM2 as termed by Rovner et al. (23), following the 3-h incubation with IL-1. We also were unable to detect the presence of the third myosin isoform, nonmuscle myosin (25), in either control or IL-1-incubated tissue. Whether this was due to the sensitivity of our assay or because only two isoforms are present in these tissues is unknown. But the fact that neither the ratio of SM1 to SM2 nor the ratio of total myosin to total protein changed suggests that IL-1 did not significantly affect thick filament content.

Our finding that IL-1 can rapidly modulate the contractile protein content of vascular smooth muscle may have important implications for the role of IL-1 in response to vascular injury. IL-1 has been shown to have a direct stimulatory effect on vascular smooth muscle cell growth (12, 13, 21). In this study, IL-1 did not affect total actin content but did significantly decrease the smooth muscle specific α-isoactin. If our speculation is correct that the increase in the γ-isoactin is primarily in the nonmuscle isoform, then this coupled with the decrease in α-isoactin would suggest that IL-1 induces a more secretory as compared with contractile phenotype in the vascular smooth muscle cell. One may then also speculate that IL-1 plays a role in regenerative and pathological processes which occur in vascular tissue.

In summary, we have demonstrated that exposure of rat aortic strips to IL-1 produces inhibition of agonist-induced contractile activity that is associated with decreased levels of MLC phosphorylation. The simplest explanation for this result is that IL-1 depresses contraction by a decrease in the activator [Ca\textsuperscript{2+}]. The results of this study also demonstrate that prolonged exposure of vascular tissue to IL-1 produces significant changes in the actin isoform profile with a decrease in the contractile-specific α-isoform and an increase in the mixed nonmuscle and muscle γ-isoform. Both of these effects may contribute to the alterations in vascular function caused by this cytokine and provide support for a role of IL-1 as a key mediator of the vascular response to tissue injury.

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