Action of Tropoelastin and Synthetic Elastin Sequences on Vascular Tone and on Free Ca^{2+} Level in Human Vascular Endothelial Cells

Gilles Faury, Stéphanie Garnier, Anthony S. Weiss, Jean Wallach, Tamás Fülöp, Jr, Marie-Paule Jacob, Robert P. Mecham, Ladislas Robert, Jean Verdetti

Abstract—The elastic properties of extensible tissues such as arteries and skin are mainly due to the presence of elastic fibers whose major component is the extracellular matrix protein elastin. Pathophysiological degradation of this protein leads to the generation of elastin peptides that have been identified in the circulation in the ng/mL to μg/mL range. Similar concentrations of an elastin peptide preparation (κ-elastin) were previously demonstrated to induce, among other biological actions, a dose- and endothelium-dependent vasorelaxation mediated by the elastin/laminin receptor and by endothelial NO production. To determine the elastin sequence(s) responsible for vasomotor activity and to learn more about possible signaling pathways, we have compared the action of different concentrations (10^{-13} to 10^{-7} mol/L) of recombinant human tropoelastin, eight synthetic elastin peptides, and a control peptide (VPVGGA) on both rat aortic ring tension and [Ca^{2+}], of cultured human umbilical vein endothelial cells. No vasoactivity could be detected for VPVGGA and for the elastin-related sequences VGVGVA, PGVGVA, and GVGVA. Tropoelastin, VGV, PGV, and VGVAPG were found to induce an endothelium- and dose-dependent vasorelaxation and to increase endothelial [Ca^{2+}], whereas PVGV and VGVA produced these effects only at low concentration (10^{-11} mol/L). A likely candidate for mediating the elastin peptide-related effects is the elastin/laminin receptor, since the presence of lactose strongly inhibited the vasoactivity associated with these compounds. Our results show that although the flanking amino acids modulate its activity, VGV seems to be the core sequence recognized by the elastin receptor. (Circ Res. 1998;82:328-336.)

Key Words: elastin ■ elastin/laminin receptor ■ vascular tone ■ endothelial cell ■ [Ca^{2+}]
present in bovine and human tropoelastin, was found to be chemotactic for both monocytes and fibroblasts.28 This same peptide and related sequences GFGVGAGVP and GLGVGAGVP were shown to be chemotactic for aortic endothelial cells.29 Other repeated elastin sequences were also found to be biologically active, including AGVPFGVG and GFGVGAGVP, which are chemotactic for fibroblasts,30 and (VPVG)\textsubscript{n}, which induces calcification in the rat.31 Furthermore, recent results indicate that the elastin peptide VGVAPG and five related shorter or frame-shifted elastin sequences (VGV, VGVA, GVGVA, PGVGVA, and VGVGVA) act in leukocytes on intracellular free Ca\textsuperscript{2+} metabolism, elastase release, released myeloperoxidase activity, or hydrogen peroxide or superoxide anion production.32

We have previously demonstrated that \( \kappa \)-elastin induces a dose- and endothelium-dependent vasorelaxation on NA-precontracted rat aortic rings, mediated by the elastin/laminin receptor and involving both NO synthase and cyclooxygenase pathways.14,15 To determine the active sequence(s) of elastin on receptor and involving both NO synthase and cyclooxygenase dose- and endothelium-dependent vasorelaxation on NA-induced precontraction (1120 mg). Statistical analysis was performed using a two-way ANOVA. In order to perform comparisons between results in the presence and in the absence of lactose, control ring tensions (with or without lactose) were normalized to 1120 mg, and the same normalization factors were then applied to ring tensions in the presence of the tested substances as described previously.35 The statistical tests were completed by Fisher's least significant difference test for paired value comparisons. Differences were considered significant at \( P<.05 \).

**Vasomotor Data Analysis**

Results are expressed as mean±SEM of aortic ring tension (mg) after a 15-minute application of each agent concentration. Initial tensions were normalized to an average value of NA-induced precontraction (1120 mg). Statistical analysis was performed using a two-way ANOVA. In order to perform comparisons between results in the presence and in the absence of lactose, control ring tensions (with or without lactose) were normalized to 1120 mg, and the same normalization factors were then applied to ring tensions in the presence of the tested substances as described previously.35 The statistical tests were completed by Fisher's least significant difference test for paired value comparisons. Differences were considered significant at \( P<.05 \).

**HUCVEC Isolation and Culture**

HUCVECs were obtained as previously described36 and were maintained, at 37°C and humid atmosphere, in fibronectin-coated dishes37 with medium 199 supplemented with Earl's salts and 22% human serum. Trypsinized cells were plated on dishes (containing 13x14-mm glass coverslips) coated with fibronectin so that the cell density was in the range of 10 500 cells/cm\textsuperscript{2}. Only first to fourth passage subconfluent cells were used in the experiments. The purity of the endothelial cell cultures was established using an antibody to von Willebrand factor.

**Loading of HUCVECs With Fura 2**

Fura 2-AM loading was performed generally according to the procedure of Thomas and Delaville.37 The culture dish was washed twice with a Tyrode's solution containing (mmol/L) NaCl 118, KCl 5.6, CaCl\textsubscript{2} 2.4, MgCl\textsubscript{2} 1.2, HEPES 10, and \( \text{d}-\text{glucose} 11 \) (pH 7.4). The dish was then filled with 0.75 mL Tyrode's solution. In the dark, 8 mL of 0.5 mmol/L fura 2-AM in DMSO was added to 250 \( \mu \)L of Tyrode's solution containing 2% BSA and 0.08% pluronic F-127 (wt/vol). This solution (258 \( \mu \)L) was then added to the culture dish (final volume, \( \approx 1 \) mL), yielding final concentrations of 4 mmol/L fura 2-AM and <1% DMSO. After a 40-minute incubation (room temperature, in the dark), the cells were washed twice and bathed with Tyrode's solution (1 mL) for 10 minutes.

**[Ca\textsuperscript{2+}], Measurements**

Fluorescence measurements were as described previously.36,37 Under low light, the HUCVEC-containing glass coverslip was removed from the culture dish and fitted vertically and diagonally in a Tyrode's solution–filled quartz cuvette (10x30 mm) with a plastic ring at the bottom that enclosed a small stir bar that mixed the solution. The coverslip was fitted so that the cells faced the dual excitation light (340 and 380 nm), and the emitted fluorescence (510 nm) was read at 90° from behind the coverslip. The cuvette was then placed at 30°C in a Deltascan 4000 spectrophotometer (Photon Technology International), and measurements were performed after a minimum of 5 minutes and before 60 minutes. The recorded signal resulted from the integrated fluorescence from all the cells facing the excitation path. [Ca\textsuperscript{2+}] was monitored for 4 minutes after the addition of elastin-related peptides to the cuvette. For investigating the inhibitory effect of lactose, fluorescence was monitored for 2 minutes in the presence of...
of lactose (10^{-4} \text{ mol/L}) alone, before the addition of the test compounds. Control experiments were performed by recording the HUVEC fluorescence alone for 5 minutes. Each measurement was stopped by the addition of 0.002% digitonin (maximum [Ca^{2+}]), followed by 45 mmol/L EGTA (minimum [Ca^{2+}]). After the autofluorescence was subtracted, [Ca^{2+}], was calculated using standard techniques, assuming that the K_{i} of fura 2 for Ca^{2+} is 224 nmol/L.^{29}

**[Ca^{2+}] Measurement Data Analysis**

For each measurement, initial [Ca^{2+}], was normalized to an average measured value (50 nmol/L). Statistical analysis was performed using a one-way ANOVA for comparison of simple effects of each elastin-related compound or of lactose. A two-way ANOVA was used to assess statistical analysis of the effect of compounds in the presence or absence of lactose. When necessary, these tests were completed by the nonparametric Mann-Whitney U test for paired value comparisons. Differences were considered significant at \( P_{<0.05} \).

**Preparation of Synthetic Elastin Peptides and Control Peptide**

All peptides were synthesized using 9-fluorenylmethoxycarbonyl chemistry. Purity of the peptides was confirmed by HPLC and by fast bombardment spectrometry. Purity and concentrations of the peptide stock solutions were verified by amino acid analysis.

**Preparation of rTE**

rTE encoded by a 2210-hp _Escherichia coli_–optimized synthetic gene was expressed and purified from bacterial extracts as described.^{37} rTE molecular mass is 63490 kD.

**Preparation of \( \kappa \)-Elastin**

Elastin peptides (\( \kappa \)-elastin) were obtained by hydrolysis of highly purified bovine ligamentum nuchae elastin with 1 mol/L KOH in 80% (vol/vol) aqueous ethanol.\(^{40}\) By adjusting the time of hydrolysis, peptides of different molecular mass can be obtained. Peptides used in these experiments had an average molecular mass of 75 kD.

**Drugs and Materials**

The following drugs and reagents were from Sigma: Krebs’ bicarbonate and HEPES-buffered solution components, NA, acetylcholine, lactose, fura 2-AM, DMSO, BSA, pluronic F-127, collagenase 1A, serum was from the Centre de Transfusion Sanguine de Grenoble. Falcon cell culture dishes were from Becton-Dickinson. The 13×14-mm glass coverslips were from Vitrone.

**Results**

**Vasorelaxant Activity of Elastin-Related Compounds**

The vasoactivity of elastin-related peptides was found to be compound and concentration dependent on aortic rings with intact endothelium (Table 1, category A). Compared with the control, the peptides VGV, PGV, GVGAPG, and rTE induced dose-dependent vasorelaxation in the NA-precontracted rat aortic rings with maximum values of 32%, 21%, 26%, and 41%, respectively (Fig 1A). Nevertheless, for each concentration for which several peptides were found to be active, no significant difference in activity could be detected between these peptides, except at 10^{-2} \text{ mol/L}, where rTE was more active than VGV (Fig 1A). Moreover, the peptide PGV was slightly active (14% vasorelaxation) at 10^{-2} \text{ mol/L} (Fig 1A). The sequences VGVA and PVGV induced a relaxation of the aortic rings only at a concentration of 10^{-2} \text{ mol/L}, with values of 14% and 19%, respectively, and no activity difference could be detected between these two peptides (Fig 1B). Finally, the peptides PGVGA, VGVGA (for which the induced aortic ring tensions presented a slightly higher variance), and VGVGA as well as the control peptide VPVGA produced tension variations of 12% maximum compared with the control, with all values not statistically significant (\( P_{>0.1} \)) (Fig 1C).

By contrast, on aortic rings without endothelium, the 10 compounds (rTE, VGV, PGV, VGVGA, PGVGA, and rTE) for concentrations demonstrated to induce a submaximal vasorelaxation. VGV, GVGAPG, and rTE were used at 10^{-8} \text{ mol/L}. Since only one concentration was found to be active in the range of the maximal effect for PGV (10^{-7} \text{ mol/L}), VGV, and PGV (10^{-11} \text{ mol/L}), this concentration was used for these compounds. The direct effect of lactose (10^{-4} \text{ mol/L}) was first studied on the NA-precontracted rat aortic rings with endothelium. Lactose (10^{-4} \text{ mol/L})–induced tension (mg) was identical to the control tension after 15 minutes but was lower than the control tension after 30 minutes and lower than the control tension and the initial lactose–induced tension after 45 minutes. At these times, the slight vasorelaxation induced by lactose was in the range of 5% compared with the control value (Fig 2 and Table 1, category C). After assessment of a second normalization of the tension values to balance the effect of lactose itself, the effect of lactose on the vasorelaxant effect induced by the active compounds (rTE, VGV, PGV, PGVGA, and rTE) for concentrations investigated on individual adherent HUVECs loaded with indo 1 or on individual adherent HUVECs loaded with indo 3 (authors’ unpublished data, 1997) was also occurring in the fura 2–loaded adherent HUVECs stimulated with 10^{-3} \text{ mg/mL} (=10^{-8} \text{ mol/L}) \( \kappa \)-elastin (Fig 3A). In these conditions, the \( \kappa \)-elastin–induced [Ca^{2+}], increase exhibited slow kinetics and an amplitude (average raw increase, 2.06-fold, or 1.73 times the control value) similar to those previously observed in pooled suspended HUVECs loaded with indo 1 (average raw increase, 2-fold). The control peptide...
The compounds found to have no activity on the vascular tone, induced an increase in HUVEC \([\text{Ca}^{2+}]\) (Fig 3B and 3C and Table 4). The effects of the elastin-related compounds on \([\text{Ca}^{2+}]\) in HUVECs (Table 2).

**TABLE 1. ANOVA Assessing the Action of Tested Elastin-Related Compounds and Lactose on Vascular Tone and \([\text{Ca}^{2+}]\), in HUVECs**

<table>
<thead>
<tr>
<th>Category Variable 1</th>
<th>Variable 2</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Elastin-related compound actions on the aortic ring tone (with endothelium)</td>
<td>Compound</td>
<td>Concentration (or time for control)</td>
</tr>
<tr>
<td></td>
<td>Mod: control, VG, VGVA, PGV, PGVGA, VPVGG, VGGVG, PGV, PGVG, PGVGA, rTE</td>
<td></td>
</tr>
<tr>
<td>B. Elastin-related compound actions on the aortic ring tone (without endothelium)</td>
<td>Compound</td>
<td>Concentration (or time for control)</td>
</tr>
<tr>
<td></td>
<td>Mod: control, VG, VGVA, PGV, PGVGA, VPVGG, VGGVG, PGV, PGVG, PGVGA, rTE</td>
<td></td>
</tr>
<tr>
<td>C. Lactose action on the aortic ring tone (with endothelium)</td>
<td>Duration</td>
<td>Lactose (10(^{-4}) mol/L)</td>
</tr>
<tr>
<td></td>
<td>Mod: 15, 30, 45 minutes</td>
<td></td>
</tr>
<tr>
<td>D. Effect of lactose on the vasodilatory action of elastin-related compounds (with endothelium)</td>
<td>Compound</td>
<td>Lactose (10(^{-4}) mol/L)</td>
</tr>
<tr>
<td></td>
<td>Mod: VGG, PGV, VGVA, PGVGA, PGVAPG, rTE</td>
<td></td>
</tr>
<tr>
<td>E. Elastin-related compound actions on ([\text{Ca}^{2+}]), in HUVECs</td>
<td>Compound</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Mod: VGG, PGV, PGVAPG, VGGVA, VPVGG, VGGVG, PGVGA, rTE, PGVGA, control</td>
<td></td>
</tr>
<tr>
<td>F. 10(^{-4}) mol/L lactose action on ([\text{Ca}^{2+}]), in HUVECs</td>
<td>Lactose</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Mod: presence, absence</td>
<td></td>
</tr>
<tr>
<td>G. Effect of lactose on the elastin-related compound–induced ([\text{Ca}^{2+}]), increase in HUVECs</td>
<td>Compound</td>
<td>Lactose (10(^{-4}) mol/L)</td>
</tr>
<tr>
<td></td>
<td>Mod: VGG, PGV, VGVA, PGVGA, PGVAPG, rTE</td>
<td></td>
</tr>
</tbody>
</table>

*Mod indicates modalities.

*Value of P < 0.05 indicates significance.

VPVGGGA (10\(^{-7}\) mol/L) had no effect on HUVEC \([\text{Ca}^{2+}]\), compared with cells with no peptide added (Fig 3B and 3C and Table 4). The effects of the elastin-related compounds on HUVEC \([\text{Ca}^{2+}]\), were then investigated at concentrations in the range of 10\(^{-9}\) to 10\(^{-7}\) mol/L for the peptides found to have no activity on the vascular tone at active concentrations for the peptides found to induce a vasorelaxation. Only rTE (10\(^{-9}\) to 10\(^{-7}\) mol/L), VGG (10\(^{-8}\) to 10\(^{-7}\) mol/L), PGV (10\(^{-8}\) to 10\(^{-7}\) mol/L), VGVA (10\(^{-11}\) mol/L), PVGV (10\(^{-11}\) mol/L), and PGVAPG (10\(^{-7}\) to 10\(^{-7}\) mol/L), ie, all the peptides found active on the vascular tone, induced an increase in HUVEC \([\text{Ca}^{2+}]\), compared with the control cells (Fig 4A and 4C and Tables 1 [category E] and 4). Nevertheless, no statistically significant difference (U test) between the \([\text{Ca}^{2+}]\), increases induced by the different active compounds could be detected. The compounds found to have no activity on the vascular tone (VPVGGGA, PGVGA, VGGVG, and GVGVA) also exhibited no significant activity on HUVEC \([\text{Ca}^{2+}]\), (Fig 3B and Tables 1 [category E] and 4).

Furthermore, compared with the control condition, application of lactose (10\(^{-4}\) mol/L) to HUVECs was not found to induce a significant change in \([\text{Ca}^{2+}]\), over a 2-minute period (Table 1, category F). However, the addition of 10\(^{-8}\) mol/L lactose inhibited the \([\text{Ca}^{2+}]\), increase induced by the active elastin-related peptides (Fig 4B and 4D and Table 4). The presence of lactose had a significant inhibitory effect for all the active elastin-related compounds tested, since the inhibition was independent of the active peptide type (Table 1, category G).

**Discussion**

Elastin or elastin-related compounds, such as the precursor molecule “tropoelastin” or its degradation products (elastin...
peptides), have been demonstrated to induce several biological effects. The results of the present study demonstrate that the whole tropoelastin molecule (rTE) as well as some elastin-related sequences induce a strong vasorelaxation on NA-precontracted adult rat aortic rings (Fig 1) and an increase in the endothelial $[\text{Ca}^{2+}]_i$ (Table 4). These results are consistent with previous findings indicating that $\kappa$-elastin produced the same effects (References 14 to 16 and authors’ unpublished data, 1997). Similar to the results obtained using $\kappa$-elastin, the absence of endothelium totally abolished the vasorelaxant action of both rTE and active synthetic elastin-related peptides (Table 2). The $\kappa$-elastin–induced endothelium– and NO-dependent vasorelaxation was shown to be mediated by the elastin/laminin receptor. Inhibition of elastin binding to the 67-kD subunit of this receptor by lactose was used to confirm the mediation of the receptor on the vasorelaxant action of $\kappa$-elastin. Lactose was shown to strongly inhibit the $\kappa$-elastin–induced vasorelaxation. In the present study, using this same approach, the actions of rTE and other vasoactive elastin-related peptides (VGV, PGV, VGVAPG, PVGV, and PGVGA) were tested.

**Figure 1.** Concentration-response curves showing the effects of elastin-related compounds after a 15-minute exposure on rat aortic ring tone. The rings were precontracted and were then perfused for 105 minutes with $10^{-6}$ mol/L NA (control). In some rings, increasing doses of elastin peptides were also added to the perfusion. The peptides added were as follows: rTE, VGV, VGVAPG, and PGV (A); PVGV and VGV (B); and VPVGGA, GVGV, VGVGVA, and PGVGA (C). Each point represents the mean of five to nine experiments. The results are expressed as tension (mg) (mean±SEM). *$P<.05$ vs control tension by least significant difference test. #$P<.05$ vs corresponding VGV-induced tension by least significant difference test. †$P<.065$ (least significant difference test) and $P<.05$ ($U$ test) vs corresponding control tension.
TABLE 2. Effect of Elastin-Related Compounds on Tone of Rat Aortic Rings Without Endothelium

<table>
<thead>
<tr>
<th>Elastin-Related Compounds</th>
<th>10^{-11} mol/L</th>
<th>10^{-8} mol/L (for PGV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1104±8</td>
<td>1081±12</td>
</tr>
<tr>
<td>VPVGGA</td>
<td>1107±9</td>
<td>1091±11</td>
</tr>
<tr>
<td>VGVAPG</td>
<td>1111±10</td>
<td>1087±24</td>
</tr>
<tr>
<td>VGGVVA</td>
<td>1112±17</td>
<td>1101±29</td>
</tr>
<tr>
<td>PGVGVA</td>
<td>1112±5</td>
<td>1096±3</td>
</tr>
<tr>
<td>GVGVA</td>
<td>1099±22</td>
<td>1073±29</td>
</tr>
<tr>
<td>PGV</td>
<td>1106±15</td>
<td>1090±18</td>
</tr>
<tr>
<td>VGV</td>
<td>1101±10</td>
<td>1083±14</td>
</tr>
<tr>
<td>PGV</td>
<td>1106±3</td>
<td>1085±10</td>
</tr>
<tr>
<td>VGV</td>
<td>1089±8</td>
<td>1102±37</td>
</tr>
<tr>
<td>rTE</td>
<td>1118±3</td>
<td>1103±21</td>
</tr>
</tbody>
</table>

Aortic rings were precontracted with 10^{-5} mol/L NA. Each value represents the mean of four experiments. The results (mean±SEM) are expressed as tension (mg).

TGVA, at active concentrations) were also strongly inhibited by the presence of 10^{-7} mol/L lactose (Table 3), indicating that their action is most likely mediated by the 67-kD subunit of the elastin/laminin receptor.

The control peptide (VPVGGA) and the elastin-related peptides VGGVVA, PGVGVA, and GVGVA were found to have no significant effect on the vascular tone. The vasorelaxant effect induced by rTE and by the peptides VGV, PGV, and VGVAPG appeared to be dose dependent (Fig 1). The 25% to 40% maximum relaxation of NA-contracted aortic rings induced by these elastin peptides is important, since its amplitude approximately corresponds to one third to half of the 70% to 80% maximum relaxation induced by the highly potent agent acetylcholine (10^{-6} to 10^{-5} mol/L) on NA-contracted aortic rings.41 Nevertheless, whereas activity of the peptides VGV, PGV, and VGVAPG directly increased with their concentration, rTE activity increased first with concentration before exhibiting a clear maximum activity at 10^{-7} mol/L and then decreasing when the concentration increased (Fig 1). This rTE activity peak at 10^{-7} mol/L, in the range of the K_d of tropoelastin binding to the elastin/laminin receptor,50 is consistent with previous findings indicating a maximum chemotactic activity of tropoelastin toward fibroblasts at the same concentration.12,13 In contrast, the effects produced by the peptides PGVG and VGVA are different from the effects of the

TABLE 3. Effect of Lactose (10^{-4} mol/L) on Action of Elastin-Related Compounds on Tone of Rat Aortic Rings With Endothelium

<table>
<thead>
<tr>
<th>Elastin-Related Compounds</th>
<th>% of Tension Variation in Absence of Lactose Compared With Control</th>
<th>% of Tension Variation in Presence of Lactose Compared With Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGVAPG</td>
<td>-18±5</td>
<td>+4±2*</td>
</tr>
<tr>
<td>PGV</td>
<td>-19±4</td>
<td>-4±1</td>
</tr>
<tr>
<td>VGV</td>
<td>-14±3</td>
<td>+0±1*</td>
</tr>
<tr>
<td>PGV</td>
<td>-21±4</td>
<td>+6±1*</td>
</tr>
<tr>
<td>VGV</td>
<td>-21±9</td>
<td>+2±3*</td>
</tr>
<tr>
<td>rTE</td>
<td>-30±9</td>
<td>+6±5*</td>
</tr>
</tbody>
</table>

Aortic rings were precontracted with 10^{-4} mol/L NA. The concentrations used were as follows: 10^{-7} mol/L PGV, 10^{-8} mol/L VGV, 10^{-8} mol/L PGVA, 10^{-8} mol/L rTE, 10^{-11} mol/L VGVA, and 10^{-11} mol/L PVGV. Each value represents the mean of five or six experiments. The results (mean±SEM), first expressed as tension (mg), were converted into percentages of tension decrease, compared with control values, after assessment of the statistical tests.

*P<.05 by two-way ANOVA. Tension measured in the presence of both elastin-related compounds and lactose was significantly higher than the tension measured in the presence of elastin-related compounds alone.

Figure 2. Action of lactose (Lac, 10^{-4} mol/L) on aortic ring tone. The rings were precontracted with NA (10^{-6} mol/L), and then the tension was recorded in the presence or absence of Lac for 45 minutes. Each point represents the mean of 9 (absence of Lac) to 17 (presence of Lac) experiments. The results are expressed as tension (mg) (mean±SEM). *P<.05 vs corresponding control tension without Lac by least significant difference test. †P<.05 vs Lac-induced tension after 15 minutes by least significant difference test.

Figure 3. Action of 10^{-3} mg/mL κ-elastin (A) and the control peptide VPVGGA at 10^{-5} mol/L (B) on [Ca^{2+}]_i in cultured HUVECs compared with the control experiment (C). Each trace is representative of six, seven, and three experiments, respectively.
In addition, the experiments performed on cultured adherent HUVECs confirmed that only the vasoactive synthetic elastin peptides and rTE produced an increase in HUVEC [Ca\(^{2+}\)], with no observed significant difference in maximal amplitude (Table 4). Furthermore, enhancement of HUVEC [Ca\(^{2+}\)], by the above-mentioned agonists was inhibited by lactose (10\(^{-3}\) mol/L), suggesting the involvement of the 67-kD subunit of the endothelial elastin/laminin receptor. The long-lasting pattern of the elastin peptide–induced [Ca\(^{2+}\)], increase (Figs 3 and 4) is similar to the \(\kappa\)-elastin–induced [Ca\(^{2+}\)], increase in suspended HUVECs and probably also results mainly from membrane Ca\(^{2+}\) channel activation (authors’ unpublished data, 1997). This slow kinetic, different from the fast [Ca\(^{2+}\)], increase (peak), involving both extracellular Ca\(^{2+}\) influx and mobilization of intracellular Ca\(^{2+}\) stores, induced by \(\kappa\)-elastin on leukocytes, matches the slow and long-lasting extracellular Ca\(^{2+}\) influx produced by \(\kappa\)-elastin in fibroblasts and smooth muscle cells. Furthermore, the slow elastin peptide–induced [Ca\(^{2+}\)], increase in HUVECs resembles the 1.3-fold [Ca\(^{2+}\)], increase induced within a few minutes by laminin in neurons, which is also mainly mediated by extracellular Ca\(^{2+}\) influx and which is involved in neuron growth.

It has previously been demonstrated that activation of the endothelial 67-kD elastin/laminin receptor by \(\kappa\)-elastin results in a dose-dependent vasorelaxation via endothelial production of NO. In the present study, it has been shown that the different active elastin peptides are probably acting through the same 67-kD receptor pathway, since both their dose-dependent vasorelaxant effect and the observed increase in endothelial [Ca\(^{2+}\)], that they induce at 10\(^{-7}\) to 10\(^{-9}\) mol/L are inhibited by lactose, an inhibitor of elastin binding to its receptor. Moreover, it has already been demonstrated that in the vascular endothelial cells there is a close correlation between [Ca\(^{2+}\)], and the amount of released NO, as well as with the resulting vasorelaxation. Therefore, it is likely that in the endothelial cells stimulated by \(\kappa\)-elastin or the different active elastin peptides, the observed NO– and dose-dependent vasorelaxation occurs concomitantly with a dose-dependent endothelial [Ca\(^{2+}\)], increase. Concerning the elastin peptides exhibiting an activity only at one concentration, it is likely that the same relation between elastin peptide–induced [Ca\(^{2+}\)], increase and vasorelaxation is applicable. At the active concentration (10\(^{-11}\) mol/L), PGVG induces an increase in endothelial [Ca\(^{2+}\)], and vasorelaxation, whereas at higher concentrations (10\(^{-8}\) mol/L), where it is inactive on vascular tone (Fig 1B), this peptide has no effect on endothelial [Ca\(^{2+}\)], (data not shown).

The peptide VGVAPG, chemotactic for fibroblasts and monocytes, and the peptides VGV and VGVA have been found to be active in both leukocytes and endothelial cells (present study). Nevertheless, the peptides GVGVA, PGVGVA, and VGVGVA, previously found to be active in leukocytes, were not found to be active in endothelial cells in our experiments. These differences could suggest that slight changes in the elastin/laminin receptor and, thus, in the sequence specificity might occur as a function of the cell type. Nevertheless, these modifications of the receptor sequence specificity could produce only minor differences, since despite numerous interspecies sequence differences, rTE is active in

<table>
<thead>
<tr>
<th>% of [Ca(^{2+})], Variation in Absence of Lactose</th>
<th>% of [Ca(^{2+})], Variation in Presence of Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPVGGA +0.3</td>
<td>ND</td>
</tr>
<tr>
<td>PGVGVA +10.15</td>
<td>ND</td>
</tr>
<tr>
<td>VGVGVA +0.5</td>
<td>ND</td>
</tr>
<tr>
<td>VGAGV +37.10*</td>
<td>+5.27†</td>
</tr>
<tr>
<td>GVGVA +7.6</td>
<td>ND</td>
</tr>
<tr>
<td>PVGV +23.8*</td>
<td>-5.6†</td>
</tr>
<tr>
<td>VGVA +56.16*</td>
<td>+31.26†</td>
</tr>
<tr>
<td>PGV +25.4*</td>
<td>-5.4†</td>
</tr>
<tr>
<td>GVGVA +40.12*</td>
<td>+14.19†</td>
</tr>
<tr>
<td>rTE +61.17*</td>
<td>+18.13†</td>
</tr>
</tbody>
</table>

ND indicates not done. Concentrations were as follows: 10\(^{-7}\) to 10\(^{-9}\) mol/L (VG, PGV, GVGVA, VGVGVA, PVGV, and VGVAPG), 10\(^{-7}\) to 10\(^{-9}\) mol/L (rTE), and 10\(^{-11}\) mol/L (VGVA and PGV). Each value represents the mean of three to seven experiments. The results (mean ± SEM) first expressed as concentration (mmol/L), were converted to the percentage of [Ca\(^{2+}\)], variation, compared with control values, after assessment of the statistical tests.

*P<.05 vs control cells (U test); †P<.05 vs cells incubated with the same elastin-related compound in the absence of lactose (two-way ANOVA).
both human and rat cells. Moreover, the number of active sequence occurrences in the rTE molecule does not seem to be a major factor related to sequence activity: PGV (26 copies in rTE) is no more active than VGV (13 copies in rTE). A more likely explanation is provided by the results obtained in leukocytes, showing that the different elastin peptides are acting via different pathways.21 For example, in leukocytes, VGVAPG and GVGVA have been demonstrated to trigger hydrogen peroxide production and to release myeloperoxidase activity, whereas only VGVAPG, and not GVGVA, was able to induce elastase release, superoxide anion production, and [Ca^{2+}] increase.22 The possibility of different peptides triggering different pathways could explain why we did not observe activity in our model for three peptides active in some pathways in leukocytes (PGVAG, VGVAG, and GGVAG); these peptides could be active in pathways and cellular events that we were not investigating.

Finally, although the flanking amino acids modulate the activity of the active sequences, VGV seems to be the core sequence interacting with the elastin receptor. The activity of this core sequence seems to decrease or at least be strongly modified (at matching concentrations) by the presence of proline residues at the N-terminus but not at the C-terminus of the peptide. However, the presence of only hydrophobic amino acids (V or A) on either side of the sequence VGV seems to decrease its activity.

An elastin peptide–induced decrease in the vascular tone is likely to induce, in vivo, an increase in the diameter of the aorta or of other elastic arteries and therefore a decrease in the resistance of these vessels. This phenomenon could be of major importance in the regulation of arterial tone or in counterbalancing the effect of hypertensive physiopathological situations involving elastin degradation, such as aging or atherosclerosis. Further in vivo studies should investigate the potential of elastin sequence–based vasorelaxant treatments.

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References


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2+ Level in Human Vascular Endothelial Cells
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Jacob, Robert P. Mecham, Ladislas Robert and Jean Verdetti

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