**α4β1 Integrin Activation of L-Type Calcium Channels in Vascular Smooth Muscle Causes Arteriole Vasoconstriction**

Kelli R. Waitkus-Edwards, Luis A. Martinez-Lemus, Xin Wu, Jerome P. Trzeciakowski, Michael J. Davis, George E. Davis, Gerald A. Meininger

**Abstract**—A pathway for the regulation of vascular tone appears to involve coupling between integrins and extracellular matrix proteins or their fragments and the subsequent modulation of ion movement across the smooth muscle cell membrane. Here, we report that the activation of L-type voltage-activated Ca\(^{2+}\) channels occurs through a novel interaction of αβ1 integrin with peptides containing the Leu-Asp-Val (LDV) integrin-binding sequence, which is found in the CS-1 region of an alternately spliced fibronectin variant. Experiments were conducted on arterioles isolated from rat skeletal muscle. Arterioles exhibited sustained concentration-dependent vasoconstriction to LDV peptides but not to Leu-Glu-Val (LEV) control peptides. The constriction was associated with increased smooth muscle cell [Ca\(^{2+}\)]\(_i\), as measured by using fura 2. The response could be inhibited with a function-blocking anti-α\(_i\) integrin antibody. Removal of the endothelium did not alter the vasoconstrictor response. Further experiments demonstrated that the vasoconstriction was abolished by the L-type Ca\(^{2+}\) channel inhibitor nifedipine and the Src family kinase inhibitor PP2. In studies of isolated smooth muscle cells using whole-cell patch-clamp methods, the L-type current was enhanced by the LDV but not LEV peptide and was blocked by PP2 or antibodies to α\(_i\) integrin. Collectively, these data indicate that activation of αβ1 integrin leads to enhanced influx of Ca\(^{2+}\) through L-type channels by activating a tyrosine kinase pathway, leading to vasoconstriction. Involvement of integrins in the modulation of vascular tone may be particularly important in vascular responses to mechanical signals, such as pressure and flow, and to tissue injury after damage to the extracellular matrix. *(Circ Res. 2002;90:1114–1121.)*

**Key Words** voltage-gated Ca\(^{2+}\) channels | vascular smooth muscle | Leu-Asp-Val | fibronectin | extracellular matrix | Src family kinases | protein kinase C

Integrins are a large family of cell-matrix and cell-cell adhesion receptors that, by virtue of their involvement in signal transduction across the cell membrane, have been shown to be involved in many cellular functions, such as proliferation, differentiation, survival, and migration. The ability of integrins to respond to soluble matrix fragments as well as insoluble extracellular matrix (ECM) proteins and to provide a link with the actin cytoskeleton has led our laboratory, as well as others, to investigate integrin participation in the control of vasomotor tone. Integrin modulation of vascular tone could be important in the vascular myogenic and flow-dependent responses, in which integrins could participate as a tension-sensing mechanism. In addition, integrins may be important vascular receptors for detecting and responding to tissue/vascular injury after damage to the ECM, resulting in exposure and formation of matricryptic sites and/or signals. We have previously shown that integrin-binding peptides that contain the arginine–glycine–aspartic acid (RGD) sequence can elicit arteriolar vasodilation or vasoconstriction by interacting with the αβ1 and αβ1 integrins, respectively. These changes in vasomotor tone appear to be mediated in large part by modulation of the Ca\(^{2+}\) current through L-type Ca\(^{2+}\) channels in vascular smooth muscle cells, such that ligation of the vitronectin receptor, αβ1, results in enhanced current through the L-type channels, and ligation of the fibronectin receptor, αβ1, results in reduced current through the L-type channels. These observations support the concept that integrins and their interactions with the matrix are an important signal transmission pathway for the regulation of vascular tone. Furthermore, these observations have led us to question whether this concept can be extended to encompass integrin-binding sequences (other than RGD) that may also be capable of altering vasomotor tone.

The integrin αβ1 is one such integrin expressed by many cell types, including leukocytes, tumor cells, and skeletal muscle and vascular smooth muscle cells. This receptor has affinity for the CS-1 region in an alternately spliced...
variant of fibronectin and the tissue injury–induced ECM protein osteopontin, as well as vascular cell adhesion molecule (VCAM)-1, which is expressed on endothelial and skeletal muscle cells. In addition, αβ integrin has been shown to bind small peptides modeled from the IIICS region of fibronectin, which contains the Leu-Asp-Val (LDV) sequence. Moreover, these peptides can competitively inhibit cell interactions with naturally occurring αβ ligands, such as fibronectin, osteopontin, and VCAM-1.

Fibronectins can be found in plasma and are a major component of the ECM, including the matrix of the vascular wall. They exist as soluble dimers and as insoluble fibrillar networks. Fibronectin diversity is generated by alternative splicing at three sites within the primary fibronectin mRNA transcript. The IIICS region of fibronectin contains a 25–amino acid sequence, including the LDV motif that binds the αβ integrin. This splice variant of fibronectin is known to be upregulated after tissue injury and wound healing and to have an increased expression of matrix metalloproteinases, which can cause degradation of fibronectin, resulting in the formation of fragments containing the CS-1 region. It has been suggested that intact fibronectin and proteolytically generated fragments of fibronectin may have differing roles. For example, intact fibronectin has been shown to serve as a chemoattractant stimulus for endothelial cells, whereas fibronectin fragments have been shown to be chemotactic for macrophages.

In the present study, we investigated the possibility that interaction of the LDV integrin–binding motif with the αβ integrin in the microvascular wall could induce a vasoactive signal to influence vasomotor tone.

Materials and Methods

Vessel Isolation

All procedures in the present study followed institutional guidelines and were approved by the Texas A&M University Animal Use and Care Committee. Male Sprague-Dawley rats (Harlan, Houston, Tex) weighing 250 to 350 g were anesthetized by intraperitoneal injection of pentobarbital sodium (100 mg/mL). Anesthesia was confirmed by the loss of spinal reflexes. After anesthesia, the right cremaster muscle was excised and pinned flat in a refrigerated (4°C) Lexan dissecting chamber. The chamber contained physiological saline solution, MOPS-PSS, as follows (mmol/L): NaCl 145.0, KCl 4.7, CaCl2 2.0, MgSO4 1.2, NaHPO4 1.0, dextrose 5.0, pyruvate 2.0, and EDTA 0.021 with 0.1 mg/mL BSA; pH was adjusted to 7.4 with NaOH. Dissected and isolated segments of first- and second-order arterioles were washed in low Ca2+-free saline solution (mmol/L) NaCl 144, KCl 5.6, CaCl2 0.1, MgCl2 1.0, NaHPO4 0.42, NaH2PO4 0.44, HEPES 10, and NaHCO3 4.17, along with 1 mg/mL BSA; pH was adjusted to 7.4 with NaOH at room temperature for 10 minutes. After cooling, 26 U/mL papain (Sigma) and 1 mg/mL diithioerythritol (Sigma) were added, and the vessels were warmed and incubated for 30 minutes at 37°C with occasional agitation. Fragments were transferred to low Ca2+-free saline solution containing 1.95 FAL-GPA U/mL collagenase (Sigma), 75 U/mL elastase (Calbiochem), and 1 mg/mL soybean trypsin inhibitor (Sigma) for 15 minutes at 37°C. These fragments were rinsed twice with low Ca2+-saline solution and gently triturated with a fire-polished Pasteur pipette to release single cells.

Patch-Clamp Techniques

Whole-cell currents were recorded by using an EPC-7 amplifier (HEKA) under the control of pClamp software (Axon Instruments). Analog-to-digital conversions were made by using a TL-1D DMA interface (Axon Instruments). Data were sampled at 5 to 10 kHz and filtered at 1 to 2 kHz by using an 8-pole Bessel filter. All experiments were performed at 22°C. Currents were recorded in the perforated-patch mode. Pipettes had resistances ranging from 1 to 3 MΩ. Perforated-patch pipettes were dipped for 2 to 3 seconds in Cs⁺ pipette solution containing (in mmol/L) CsCl 110 and tetraethylammonium (TEA) chloride 20 (to block endogenous K⁺ currents), EGTA 10, MgCl2, 2, HEPES 10, and CaCl2 1 (pH adjusted to 7.2 with CsOH). Pipettes were back-filled with the same solution containing 240 μg/mL amphotericin B. To record whole-cell current through the Ca2⁺ channel, Ba2⁺ (20 mM/mL) was used as the charge carrier, and the extracellular solution contained (in mmol/L) BaCl2 20, choline chloride 124, HEPES 10, and d-glucose 15 (pH adjusted to 7.4 with TEA-OH). Raw current values were normalized to cell capacitance (an index of cell size) and were expressed as current density (pA/pF) for comparisons. Cell capacitance ranged from 4 to 16 pF. Soluble peptides and antibodies (eg, LDV, LEV, and anti-α integrin) were applied locally to cells by using picospritzer pipettes (General Valve Corp).

Removal of Endothelium From Isolated Arterioles

The removal of the endothelium was necessary to determine a potential role for the endothelium in response to LDV-containing peptides. Deendothelialization was accomplished by using a non-ionic chemical detergent, CHAPS (4%, Sigma Chemical Co), as previously described.

Ca2⁺ Measurement

Vascular smooth muscle cytosolic Ca2⁺ concentration ([Ca2⁺]) was measured in isolated arterioles by using the Ca2⁺ fluorescence indicator fura 2 (Molecular Probes) as previously reported by this laboratory. Equilibrated arterioles were incubated in a solution of 2.0 μM fura 2-AM in 0.05% dimethyl sulfoxide and 0.01% pluronic F-127 for 45 minutes at 37°C. After this loading period, arterioles were rinsed with fresh PSS and allowed to equilibrate for an additional 30 minutes at 34°C. Changes in [Ca2⁺], were recorded with a microfluorometer system with the use of a photomultiplier tube. To simultaneously record diameter during the [Ca2⁺] measurement, transillumination light was passed through an orange absorption glass filter (OG-590) that transmitted light >575 nm. Thus, fura 2 measurements were not affected.

Cell Isolation Procedure

Cremaster muscles were excised and pinned flat for dissection at 4°C in Ca2⁺-free saline solution (mmol/L) NaCl 147, KCl 8.6, MOPS 3.0, MgSO4 7H2O 1.17, NaHPO4 1.2, d-glucose 5.0, pyruvate 2.0, and EDTA 0.021 with 0.1 mg/mL BSA; pH was adjusted to 7.4 with NaOH. Dissected and isolated segments of first- and second-order arterioles were washed in low Ca2+-free saline solution containing (in mmol/L) NaCl 144, KCl 5.6, CaCl2 0.1, MgCl2 1.0, NaHPO4 0.42, NaH2PO4 0.44, HEPES 10, and NaHCO3 4.17, along with 1 mg/mL BSA; pH was adjusted to 7.4 with NaOH at room temperature for 10 minutes. After cooling, 26 U/mL papain (Sigma) and 1 mg/mL diithioerythritol (Sigma) were added, and the vessels were warmed and incubated for 30 minutes at 37°C with occasional agitation. Fragments were transferred to low Ca2⁺ saline solution containing 1.95 FAL-GPA U/mL collagenase (Sigma), 75 U/mL elastase (Calbiochem), and 1 mg/mL soybean trypsin inhibitor (Sigma) for 15 minutes at 37°C. These fragments were rinsed twice with low Ca2⁺ saline solution and gently triturated with a fire-polished Pasteur pipette to release single cells.

Peptide and Antibody Treatments

The LDV-containing peptide Glu-Ile-Leu-Asp-Val-Ser-Pro-Thr and LEV-containing peptide Glu-Ile-Leu-Glu-Val-Ser-Pro-Thr (Peninsula Labs) were solubilized in PSS. A cumulative concentration-response relationship was obtained by abluminal addition of LDV or LEV (0.21 μmol/L to 0.21 mmol/L) to the vessel bath. Diameter was recorded once per minute for up to 7 minutes to ensure that a steady-state diameter was reached. Diameter changes generally were at steady state within 2 to 3 minutes.

Patch-Clamp Techniques

Whole-cell currents were recorded by using an EPC-7 amplifier (HEKA) under the control of pClamp software (Axon Instruments). Analog-to-digital conversions were made by using a TL-1D DMA interface (Axon Instruments). Data were sampled at 5 to 10 kHz and filtered at 1 to 2 kHz by using an 8-pole Bessel filter. All experiments were performed at 22°C. Currents were recorded in the perforated-patch mode. Pipettes had resistances ranging from 1 to 3 MΩ. Perforated-patch pipettes were dipped for 2 to 3 seconds in Cs⁺ pipette solution containing (in mmol/L) CsCl 110 and tetraethylammonium (TEA) chloride 20 (to block endogenous K⁺ currents), EGTA 10, MgCl2, 2, HEPES 10, and CaCl2 1 (pH adjusted to 7.2 with CsOH). Pipettes were back-filled with the same solution containing 240 μg/mL amphotericin B. To record whole-cell current through the Ca2⁺ channel, Ba2⁺ (20 mM/mL) was used as the charge carrier, and the extracellular solution contained (in mmol/L) BaCl2 20, choline chloride 124, HEPES 10, and d-glucose 15 (pH adjusted to 7.4 with TEA-OH). Raw current values were normalized to cell capacitance (an index of cell size) and were expressed as current density (pA/pF) for comparisons. Cell capacitance ranged from 4 to 16 pF. Soluble peptides and antibodies (eg, LDV, LEV, and anti-α integrin) were applied locally to cells by using picospritzer pipettes (General Valve Corp).

α integrin Expression by Arteriolar VSMA In Situ

Vessels were fixed with 2% paraformaldehyde-PSS for 20 minutes. After two glycine-PSS washes, the vessels were treated for 2 hours with an anti-α monoclonal antibody (MAB1936Z), the anti-
major histocompatibility complex class I antigen monoclonal antibody (Seikagaku), or FITC-conjugated secondary antibody alone. Primary antibodies were diluted (25 μg/mL) in buffer with 2% goat serum. Vessels were washed with PBS and with FITC-conjugated goat anti-mouse antibody (1 μg of antibody, DAKO Corp). Fluorescent images were acquired by use of the Scanalytics Cellscan imaging system (Scanalytics, CSP, Inc). Alternatively, in some experiments, the isolated arterioles were not fixed before incubation with the anti–α4 monoclonal antibody. In these experiments, confocal images (Meridian Ultima-Z 312, Laser Scanning Confocal System, Meridian Instruments) of the arterioles were obtained to visualize α4 integrin distribution. This was followed by a carboxyfluorescein dye exclusion imaging technique to visualize the vascular smooth muscle cells. The two sets of images were then overlaid to show α4 integrin distribution in relation to single smooth muscle cells.

**Experimental Protocols**

Arteriolar diameter changes to peptides were quantified as a percentage of constriction normalized to starting diameter. Maximal constriction was set as the inner luminal diameter after the addition of 10 μmol/L phenylephrine. After each dose, arteriolar diameter was measured each minute for 7 minutes or until a steady-state diameter was maintained for 2 to 3 minutes. The bath was then washed with fresh PSS after completion of the concentration-response curve to remove any remaining peptide or protein fragments. The vessel was allowed to reequilibrate and return to the baseline diameter. The vessel was then incubated with either 50 μg/mL of the β3 monoclonal function–blocking antibody F11 (Pharmingen) or 25 μg/mL of MAB1396Z, the α4 monoclonal function–blocking antibody (Chemicon). The vessel was pretreated with the antibodies for 15 minutes, after which time the concentration-response curve was repeated to determine the effects of each respective antibody.

To investigate the involvement of Src family tyrosine kinases, isolated arterioles were incubated for 20 minutes with the Src family kinase inhibitor PP2 (1 μmol/L) or the inactive analogue PP3 (1 μmol/L) (Calbiochem). Similarly, isolated vascular smooth muscle cells were also incubated with PP2 (1 μmol/L for 20 minutes) to inhibit the Src family kinases or the inactive analogue PP3 (1 μmol/L for 20 minutes) as a control. A role for protein kinase C was investigated by using the protein kinase C (PKC) inhibitor calphostin C (Sigma). Isolated arterioles or isolated vascular smooth muscle cells were incubated with 1 μmol/L calphostin C for 30 minutes. The arteriolar vasomotor responses or cellular electrophysiological responses to LDV were recorded before and after treatment with the inhibitors.

**Data Analysis**

All data are expressed as mean±SEM. Analyses of time-matched responses were performed by ANOVA for two repeated factors, followed by post hoc tests for differences or by an independent
2-tailed Student t test. In all statistical analyses, a value of $P < 0.05$ was used as the level of probability indicating significance.

**Results**

After addition of the LDV peptide, arterioles exhibited a slowly developing monophasic constriction that was maintained in the presence of the peptide. The constriction reached steady state within 7 to 8 minutes and was reversed by rinsing with fresh PSS, with the arterioles returning to their baseline diameter (Figure 1A). The concentration-response relationships illustrating the vasoactive responses produced by the addition of LDV and its control peptide, LEV, are shown in Figure 1B. The control peptide, LEV, had no effect on vasomotor tone. LDV-containing peptides produced a significant concentration-dependent vasoconstriction ($n=7$, $P<0.05$) and, at the highest concentration ($2.1 \times 10^{-4}$ mol/L), resulted in a constriction of 19%.

To determine whether the release of an endothelial factor was contributing to or modulating the LDV-induced vasoconstriction, responses to LDV were compared in arterioles before and after removal of the endothelium (Figure 1C). There was no significant difference between the concentration-response curves to LDV in the presence or absence of the endothelium ($n=6$).

Specificity of the constrictor response to LDV for the $\alpha_4$ integrin was determined by pretreatment of the arterioles for 15 minutes with the anti-$\alpha_4$ (MAB1396Z) monoclonal function–blocking antibody (Figure 2A). The vasoconstrictor response to LDV was abolished by anti-$\alpha_4$ pretreatment ($P<0.05$). For comparison, pretreatment of the arterioles with an anti-$\beta_3$ integrin function–blocking antibody (F11) had no significant effect on LDV-induced vasoconstriction (Figure 2B), indicating the specificity of the $\alpha_4$ antibody. Previously, this $\beta_3$ antibody was used to block the vasodilatory effect of RGD peptides. 2

The presence of the $\alpha_4$ integrin in isolated arterioles was demonstrated by fluorescence immunohistochemical labeling of intact arterioles (Figure 3). Specific punctate staining (single-headed arrow) for $\alpha_4$ integrin was visible along the vascular smooth muscle cell margin. Vascular smooth muscle cells were oriented perpendicular to the long axis of the vessel (double-headed arrows) (Figure 3A). The secondary antibody alone failed to show any specific labeling (Figure 3B). Likewise, anti-rat major histocompatibility complex class I antibody failed to label the arteriole in a specific fashion (data not shown).

Measurements of $[Ca^{2+}]_i$ in arterioles loaded with fura 2 (Figures 4A and 4B) indicated that the LDV-induced vasoconstriction was associated with an increase in vascular smooth muscle $[Ca^{2+}]_i$. After the addition of $1 \times 10^{-4}$ mol/L LDV, the arterioles were observed to constrict by $\approx 18\%$, whereas $[Ca^{2+}]_i$ was increased by $\approx 10\%$. To determine whether the LDV vasoconstriction involved $Ca^{2+}$ entry through voltage-operated L-type channels, the arterioles were treated with nifedipine (1 $\mu$mol/L) before the addition of
LDV-containing peptides. Nifedipine completely abolished the LDV-induced vasoconstriction (Figure 5).

Direct evidence of the involvement of L-type Ca^{2+} channels was obtained by using the patch-clamp technique. Inward Ba^{2+} currents through L-type Ca^{2+} channels were obtained by using voltage ramps (−100 to 80 mV, duration 200 ms) or voltage steps (−80 to 80 mV in 10-mV increments, duration 100 ms). The whole-cell Ba^{2+} currents

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**Figure 3.** In situ α₄ integrin expression on rat cremaster vascular smooth muscle cells. A, Isolated cremaster skeletal muscle arterioles were labeled with the anti-α₄ integrin monoclonal function–blocking antibody MAB1396Z. The double-headed arrows indicate the long axis of the isolated arteriole. The green fluorescent signal representing the α₄ label is indicated by the smaller single arrow. α₄ labeling is visibly distributed along vascular smooth muscle cell margins (larger single arrows). Cells, shown in red, are oriented perpendicular to the vessels axis of orientation. B, Artroioles treated with the secondary antibody alone show no specific labeling.

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**Figure 4.** Effect of LDV on arteriolar vascular smooth muscle [Ca^{2+}]. A, Change in diameter expressed as a percentage of control after addition of LDV as a function of time in minutes (single experiment). B, Changes in diameter (percentage of control) and vascular smooth muscle [Ca^{2+}] (percent increase in the 340/380 ratio). Data are expressed as mean±SEM. *P<0.05 vs basal diameter or [Ca^{2+}].
Figure 5. Effect of nifedipine (10^{-6} mol/L) on the vasoconstrictor response to LDV (2.1×10^{-4} mol/L). A, Changes in arteriolar diameter after treatment with peptides containing LDV both before and after inhibition of L-type channels with nifedipine. Black bar illustrates passive arteriolar diameter obtained in the presence of adenosine (10^{-3} mol/L) and Ca^{2+}-free PSS containing EGTA. B, Difference in arteriolar diameter expressed as percent change in basal diameter. Data are expressed as mean±SEM. *P<0.05 vs basal diameter.

Discussion

In the present study, we have shown that the LDV peptides that are synthesized to mimic the CS-1 (alternately spliced) region of fibronectin cause vasoconstriction in isolated resistance arterioles. The vasoconstriction induced by LDV peptides is unaffected by removal of the endothelium and is inhibited by blocking the α4 integrin subunit with a monoclonal function-blocking antibody, supporting an interaction with the vascular smooth muscle cell α5β1 integrin. The mechanism of the vasoconstriction involves an increase in vascular smooth muscle Ca^{2+} that is dependent on the influx of Ca^{2+} through voltage-operated L-type channels. These data collectively support a role for the regulation of Ca^{2+} entry by integrin-linked signaling pathways.

Evidence of a link between integrins and the regulation of vascular tone comes from several laboratories that have reported that peptides containing the RGD integrin-binding motif have the ability to alter vascular tone. The rationale for assuming a vasoregulatory role is that many of the integrin-linked cell signaling pathways are similar to those linked to the regulation of smooth muscle contractile function. The cell signaling pathways linked to control of the influx of Ca^{2+} are good examples. At present, αβ, αβ integrin ligands have both been reported to alter vasomotor tone in arterioles and to modulate the influx of Ca^{2+} through L-type channels. The αβ integrin appears to be the major integrin linked cell signaling pathways to vasomotor function, and produces vasoconstriction. Recent investigations of the mechanism responsible for coupling the L-type channel to the αβ integrin indicate that a tyrosine phosphorylation cascade involving focal adhesion kinase and c-Src is important. In the present study, we report another integrin and integrin-binding motif, αβ and LDV, respectively, that are capable of enhancing the

peaked at 30 mV (range −3.9 to −8.9 pA/pF) and were blocked by nifedipine (1 μmol/L). The application of peptides containing LDV (100 μmol/L) resulted in an enhanced current through the L-type channels (Figure 6A) and caused downward displacement of the current-voltage relationship (Figure 6B). Modulation of the current over the entire voltage range indicated that the LDV peptides did not shift the sensitivity of the channel to voltage. Figure 6C shows a time course of peak Ba^{2+} currents after the application of LDV compared with basal current during the course of an experiment. To address the specificity of the effect of LDV-containing peptides on the L-type channel current, studies were repeated in the presence of peptides containing the substituted sequence, LEV (100 μmol/L). LEV had no effect on L-type current (Figure 8). Further experiments in the presence of a function-blocking antibody to the α4 integrin (10 μg/mL) indicated that the enhancement of L-type channels by LDV was inhibited by preincubation of the vascular smooth muscle cell for 1 minute with the antibody to α4 integrin (20 μg/mL). The soluble α4 antibody alone had no significant effect on the current (Figure 8).

Additional experiments to address the mechanism for the effects of LDV indicated that the vasoconstrictor response and enhancement of L-type current were tyrosine kinase dependent, inasmuch as both were inhibited by the Src family kinase inhibitor PP2 but were not affected by the inactive analogue, PP3, used as a control (Figures 7 and 8). By comparison, inhibition of PKC was unable to inhibit the vasoconstrictor response or the enhancement of L-type current in response to LDV. However, PKC inhibition did attenuate basal vascular tone and basal current (Figures 7 and 8). These effects of PKC inhibitors are consistent with a role for PKC in the regulation of the L-type Ca^{2+} channel but not in the regulation of the channel by αβ integrin ligands.
influx of Ca$^{2+}$ through L-type channels and causing vasoconstriction. Furthermore, the link between $\alpha_4\beta_1$ and the L-type channel involves an Src family kinase cascade similar to the $\alpha_5\beta_1$ integrin. 14

The ability of integrins to play a role in the regulation of arteriolar vasomotor tone could be of particular relevance in local vasoregulatory responses to mechanical forces, such as those imposed by changes in intravascular pressure or fluid shear stress. Thus, an involvement of integrins in the vascular myogenic response and flow-dependent vascular responses may be at the level of the putative “mechanosensor.” Because integrins link the ECM to the cellular actin cytoskeleton at membrane focal contacts/dense plaques and because these sites act as a site for the assembly of a number of signaling pathway components, they are strategically placed to act as a mechanotransducing element. The dependence of the vascular myogenic response on the influx of Ca$^{2+}$ through voltage-operated L-type channels and the ability of LDV to enhance Ca$^{2+}$ entry and cause vasoconstriction could be interpreted to indicate that the $\alpha_4\beta_1$ integrin is involved in the regulation of myogenic tone.

Presumably, it would be an interaction of the $\alpha_4\beta_1$ integrin with fibronectin or osteopontin that would be important for mechanotransduction, because these matrix ligands are natural ECM ligands for this integrin. However, it is not clear whether the alternately spliced CS-1–containing forms of fibronectin or osteopontin are normally present in the vascular wall matrix. Another intriguing possibility is that the $\alpha_4\beta_1$ integrin may be modulating myogenic function through cell-cell interactions involving VCAM-1 rather than through a cell ECM. Although VCAM-1 is known to be expressed by vascular smooth muscle, most evidence suggests that its expression is important during vascular development, after vascular wall injury, and after stimulation by various cytokines. Consequently, the importance of its expression in normal vascular smooth muscle cells is less certain.

We have previously hypothesized that the vasomotor responses mediated by integrins may be particularly impor-
tant during tissue and/or vascular injury. This hypothesis is based on the existence of biologically active cryptic sites within ECM molecules (matricryptic sites) that are capable of interacting with integrins.9 These sites are normally not exposed until such time that an injury occurs, during which time ECM enzymatic breakdown, denaturation, multimerization, and/or adsorption leads to the exposure or production of fragments (matricryptins) containing matricryptic sites.9–12 These injury-derived signals would then participate in a variety of injury/repair events, including alterations in vasomotor tone. Regarding the α4β1 integrin, several observations point to a role in tissue injury/repair processes. First, the α4β1 integrin is upregulated on vascular smooth muscle cells15 during injury. Second, expression of the alternately spliced CS-1–containing variants of fibronectin,44–46 osteopontin,19 and VCAM-1 are increased after injury.40–42 Thus, interactions among these molecules may take on more importance during vascular responses to injury.

In conclusion, the present study indicates a unique ability of the α4β1 integrin to alter vasomotor tone through the modulation of membrane Ca2+ conductance. We propose that interactions between the α4β1 integrin and the ECM or other adhesion molecules are important for vascular control, particularly in tissue-injury responses. Alterations in the α4β1 integrin and ECM protein expression as well as enzymatic liberation of matricryptins may be important factors in the induction of vascular dysfunction after cardiovascular injury.

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