Integrins and mechanotransduction of the vascular myogenic response

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1Department of Medical Physiology and 2Department of Pathology and Laboratory Medicine, Cardiovascular Research Institute, Texas A&M University System Health Science Center, College Station, Texas 77845-1114; and 3Department of Human Biology, Royal Melbourne Institute of Technology, Bundoora, Victoria 3083, Australia

Davis, Michael J., Xin Wu, Timothy R. Nurkiewicz, Junya Kawasaki, George E. Davis, Michael A. Hill, and Gerald A. Meininger. Integrins and mechanotransduction of the vascular myogenic response. Am J Physiol Heart Circ Physiol 280: H1427–H1433, 2001.—This review summarizes what is currently known about the role of integrins in the vascular myogenic response. The myogenic response is the rapid and maintained constriction of a blood vessel in response to pressure elevation. A role for integrins in this process has been suggested because these molecules form an important mechanical link between the extracellular matrix and the vascular smooth muscle cytoskeleton. We briefly summarize evidence for a general role of integrins in mechanotransduction. We then describe the integrin subunit combinations known to exist in smooth muscle and the vascular wall matrix proteins that may interact with these integrins. We then discuss the effects of integrin-specific peptides and antibodies on vascular tone and on calcium entry mechanisms in vascular smooth muscle. Because integrin function is linked to the cytoskeleton, we discuss evidence for the role of the cytoskeleton in determining myogenic responsiveness. Finally, we analyze evidence that integrin-linked signaling pathways, such as those involving protein tyrosine phosphorylation cascades and mitogen-activated protein kinases, are required for myogenic tone.

pressure-induced constriction; focal adhesion; protein tyrosine phosphorylation; cytoskeleton; focal adhesion kinase; Src kinase; extracellular matrix; dense plaque

THE VASCULAR MYOGENIC RESPONSE is the rapid and maintained constriction of a blood vessel in response to pressure elevation. At physiological pressure levels, the vascular myogenic response produces a tonic arteriolar constriction necessary for the action of vasodilators. The myogenic response participates in local regulation of blood flow and protects dependent capillary beds from large increases in hydrostatic pressure induced by postural changes (10). Numerous studies also implicate this response in the vascular pathologies associated with hypertension and diabetes. Several signaling mechanisms that contribute to the myogenic response are now understood: the response is independent of the endothelium and requires both calcium entry and calcium-sensitization processes in vascular smooth muscle (VSM) (see Refs. 7, 10, 21, and 48 for reviews). Although several lines of evidence point to an underlying, tension-sensing mechanism by VSM (10, 26), the specific sensing sites, if any, are unknown. There has been recent interest in the possible role of adhesion molecules in this process, particularly integrins, because they form an important mechanical link between the extracellular matrix and the cytoskeleton. The focus of this review is to summarize what is currently known about the role of integrins in the vascular myogenic response.
ROLE FOR INTEGRINS IN MECHANOTRANSDUCTION

Integrins represent a class of membrane-spanning glycoproteins that link the extracellular matrix (ECM) with the cytoskeleton (CSK). Integrins are composed of $\alpha$-$\beta$ heterodimers with extracellular domains that bind to ECM and short cytoplasmic tails that associate with focal adhesion proteins (66). Stress applied through integrin-specific adhesion sites increases cytoskeletal stiffening (68), activates second messenger formation (37), and induces tyrosine phosphorylation of proteins anchored to the CSK (56). Considerable evidence suggests that integrins can transduce mechanical force across the plasma membrane and initiate intracellular signaling (3, 55, 65). An important question that remains is whether integrin signaling is involved in the myogenic response.

Integrin engagement by multivalent ligands, including ECM proteins, induces integrin clustering and recruitment of CSK proteins to the focal adhesion (6, 39). In cultured cells, focal adhesions are structures that play critical roles in cell attachment to substrate and locomotion because they also represent anchoring points for the CSK. The analogous structure for the in situ VSM cell appears to be the dense plaque (dense body). Contractile proteins attach to the plasma membrane at dense plaques, which are arranged in a distinct pattern at the cell surface and change orientation as the cell shortens (12, 59). Dense plaques contain many or most of the same proteins found in focal adhesions of other cell types (15), and some of these, including focal adhesion kinase (FAK) and paxillin, become phosphorylated during smooth muscle contraction (51). Dense plaques also contain nonreceptor protein tyrosine kinases (PTK) that could potentially phosphorylate proteins involved directly or indirectly in contraction (64). To build a compelling case for integrin involvement in the myogenic response, it is necessary to discuss the role of the ECM-integrin-CSK-PTK axis in mechanotransduction of pressure by VSM.

VASCULAR SMOOTH MUSCLE INTEGRINS

The VSM cell is embedded in a complex three-dimensional network of ECM proteins, including type I, III, IV, V, and VI collagens, fibronectin, vitronectin, thrombospondin, elastin, tenascin, osteopontin, and several types of laminin (15). At least 11 of the 22 known integrin subunit combinations occur in VSM: $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_5\beta_3$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, and $\alpha_6\beta_5$ (15). The predominance of $\alpha_1\beta_1$, $\alpha_5\beta_1$, $\alpha_5\beta_3$, and $\alpha_\beta_3$-integrins in VSM suggests there are extensive interactions with collagens, laminin, and fibronectin in the basement membrane and interstitial matrix (15). It is well documented that chronic stretch of VSM alters matrix expression through an integrin-dependent process (25), and that integrin and matrix production are increased in hypertension (47). Much less is known regarding the role of matrix and integrins in acute control of VSM force production, i.e., over the time course of a myogenic contraction. However, the ECM can directly transmit force to the VSM cell layer (18), and therefore acute increases in transmural pressure produce increased circumferential wall tension and increased longitudinal stress. Myogenic activation and contraction of VSM generates a force to partially offset the increase in wall tension, but this, in turn, alters the state of the ECM and the force distribution between ECM and VSM (48). The possible impact of these micromechanical changes on integrin-mediated signal transduction is unknown.

EFFECTS OF INTEGRIN-SPECIFIC PEPTIDES ON VASCULAR TONE

The most direct evidence for an integrin role in the myogenic response derives from observations that peptides containing integrin-specific amino acid sequences are potently vasoactive. Four predominant VSM integrins all contain the Asp-Gly-Asp (RGD) recognition site (15). This sequence is found in many vascular wall proteins, including collagen and fibronectin. In isolated rat cremaster arterioles, RGD-containing peptides cause a transient constriction followed by a sustained dilation (41). RGE, the appropriate control peptide, is not vasoactive. Vasodilation potency is enhanced by peptide cyclization, which implicates $\alpha_\beta$-integrin involvement (52). Indeed, an anti-$\beta_3$-integrin antibody attenuates the vasodilation to cRGD, whereas proteolyzed fragments of type I collagen, which contain exposed RGD residues, induce a vasodilation that is also blocked with $\beta_3$-antibody (9, 41). The arteriolar dilation to cyclo-RGD peptide is preceded by a decrease in VSM intracellular calcium concentration ([Ca$^{2+}$]), suggesting that the peptide acts by interfering with a calcium entry mechanism (8). Additional pharmacological and electrophysiological evidence implicates K$^+$ and Ca$^{2+}$ channels in this response (54, 74).

Whereas the preceding observations suggest that integrin ligands may selectively inhibit myogenic tone, dilation to RGD peptides is also observed in rat aortic strips precontracted with norepinephrine (29) or angiotensin II (57). The relaxation in aorta is endothelium independent and is associated with an increase in cGMP (29). Surprisingly, RGD peptides produce constriction of isolated rat renal afferent arterioles, associated with an increase in VSM [Ca$^{2+}$], (77). The constrictions produced by pressure and RGD peptide are additive, suggesting that different downstream signaling mechanisms are involved. Alternatively, RGD-mediated vasodilation and vasoconstriction might be mediated by different integrins. Although RGD-mediated constriction of afferent arterioles was observed under conditions in which nitric oxide synthesis was inhibited, release of vasoconstrictors from the endothelium cannot be ruled out (77). Indeed, RGD peptides do have effects on endothelium, including stimulation of endothelin production to produce maintained constriction of skeletal muscle arterioles (40) and stimulation of cyclooxygenase-dependent vasodilation in porcine coronary arterioles (38). Interestingly, RGD-containing peptides and integrin antibodies also interfere with endothelium-dependent, flow-induced dilation and con-
striction (32, 42, 63), responses that also involve mechanotransduction processes.

It should be noted that at least one other integrin-specific binding sequence has been identified in VSM. LDV-containing peptides (which interact with \( \alpha_5\beta_1 \)) cause constriction of rat cremaster arterioles (67). This constriction is endothelium-independent and blocked by pretreatment with an \( \alpha_5\)-integrin function-blocking antibody. Collectively, experiments with integrin-specific peptides point to the involvement of various VSM integrins in control of vascular tone, but a link to a specific role in myogenic signaling is currently tenuous.

INTEGRIN-REGULATION OF CALCIUM ENTRY

A second line of evidence for an integrin role in the myogenic response is based on the overlap between calcium-signaling and integrin-signaling pathways in VSM. Interactions between vascular cell integrins and their appropriate ligands can initiate calcium influx (2, 75). It is well known that calcium influx is essential for sustaining myogenic tone in almost all vessels studied (10), because antagonists of the L-type calcium channel, e.g., dihydropyridines, rapidly inhibit myogenic tone. PTK inhibitors such as genistein and herbimycin A attenuate depolarization-induced tone (16, 65), myogenic (basal) tone (34, 49), and agonist-induced tone (11, 22). Genistein also inhibits the rise in VSM \([\text{Ca}^{2+}]_i\) induced by KCl depolarization (16), suggesting it acts on a calcium entry mechanism. More substantial evidence is that L-type currents in rabbit ear artery (69) and portal vein (30) are inhibited by genistein and enhanced by tyrosine phosphatase inhibition (72).

With regard to regulation of calcium entry, ligands of the \( \alpha_5\beta_3 \)-integrin inhibit dihydropyridine-sensitive calcium currents in arteriolar myocytes from skeletal muscle (74). This is consistent with the observation that \( \alpha_5\beta_3 \)-ligands inhibit myogenic tone in those vessels (41). In contrast, ligands of the \( \alpha_5\beta_1 \)-integrin substantially enhance L-type calcium current (74) and initiate \([\text{Ca}^{2+}]_i\) increases (unpublished observations) in arteriolar VSM cells. Whether force transduced through \( \alpha_5\beta_3 \) or \( \alpha_5\beta_1 \)-integrins leads to modulation of calcium current or calcium entry is not yet known. However, the interaction of \( \alpha_5\beta_1 \) and the L-type calcium channel may explain how RGD peptides produce transient constrictions of skeletal muscle arterioles (41). Thus multiple lines of evidence suggest that the ECM exerts constitutive control over the major calcium-permeable ion channel in VSM and suggest that physiological regulation of calcium signaling by integrins occurs in blood vessels.

By what mechanism might integrins regulate calcium influx in VSM? Following integrin clustering, kinases such as FAK, sarcoma virus tyrosine kinase (Src), and phospholipase C-\( \gamma \) (PLC-\( \gamma \)), and other signaling and structural proteins, such as Rho GTPase, paxillin, and vinculin, are recruited to the ECM-integrin binding site (3, 66). Subsequently, phosphorylation cascades are initiated. In arteriolar myocytes, engagement and clustering of \( \alpha_5\beta_1 \)-integrins are necessary to produce the enhancement in the calcium current described above (74). This process is prevented by broad-spectrum PTK inhibitors but also by a c-Src-specific inhibitory peptide or c-Src antibody. Furthermore, basal L-type current is increased by intracellular application of constitutively active Src kinase (73). Similar observations have been made in visceral (23) and vascular (70, 71) smooth muscle in response to platelet-derived growth factor. However, with respect to integrins, antibodies to other components of the focal adhesion complex interfere with \( \alpha_5\beta_1 \) regulation of calcium current (73). These include anti-FAK, anti-paxillin, and antibodies to other focal adhesion or CSK proteins containing SH3 or SH2 domains, which are recognition sites necessary for the assembly of these proteins in the focal adhesion complex. Control antibodies had no significant effect on current (73). Collectively, this evidence strongly suggests that c-Src, and perhaps other tyrosine kinases, constitutively regulate L-type calcium current in VSM. Thus one of the key players in integrin signaling may be a regulator of \([\text{Ca}^{2+}]_i\), the primary trigger for VSM contraction.

ROLE OF CSK IN MYOGENIC RESPONSE

Because the integrin-dense plaque region of the VSM cell represents the mechanical junction between the extracellular and intracellular environments and an assemblage site for the collection of many signaling pathways and CSK components, it is a likely site for a putative mechanosensitive “element.” This element is probably not a single protein but an assemblage of proteins with which interactions form a functional mechanosensor. The role of the CSK in generation of basal vascular tone constitutes a third line of evidence that integrins may be involved in the myogenic response.

A prevailing model of the CSK (the tensegrity model) describes the CSK as a highly organized, three-dimensional syncytium of compression-resistant struts (microtubules) suspended among various elastic elements (intermediate filaments and actin filaments) (24). Thus changes in any element can potentially affect contractile function and blood vessel responsiveness to stimulation. Knowledge of the structural properties of the cytoskeleton support the view that the CSK is the principal force-generating and stress-bearing part of the cell. Upon exposure to external mechanical stress, the CSK will restructure itself (60), presumably to adapt to the force and/or offset an intracellular tension-bearing or tension-sensing element.

The tensegrity model predicts that microtubules would normally oppose contractile shortening during activation of the contractile filaments. Accordingly, disruption of these microtubule struts would permit more efficient shortening of VSM cells and subsequent transmission of this generated force to wall shortening events. Recent work has demonstrated that the state of microtubule polymerization can significantly affect VSM tone. Agents that disrupt the polymerized state of microtubules cause a significant increase in myogenic...
tone of skeletal muscle arterioles (53). In conduit arteries, microtubule depolymerization enhances contractile responsiveness to agonists (28, 50, 58). Conversely, taxol, an agent that stabilizes microtubules, is reported to have no effect on contractile responsiveness or even to inhibit the generation of spontaneous tone (1, 53). Microtubule depolymerization may also produce an alteration in the passive mechanical characteristics of the CSK, which could indirectly alter active force development. Although this remains a possibility, definitive experimental evidence to support a mechanism based on the mechanical stiffness of the microtubules is lacking.

Microtubule disruption might also alter vascular tone by an effect on cellular signal transduction. Paul et al. (50) reported an increase in VSM [Ca\textsuperscript{2+}] of porcine coronary arteries following disruption of microtubules. However, in intact skeletal muscle arterioles, there appears to be no change in VSM [Ca\textsuperscript{2+}], following depolymerization, which would explain the enhanced development of tone (53). The reasons for these opposing observations are presently unclear but may relate to origin of the arteries investigated. Recent evidence from the laboratories of GA Meininger and RC Webb (unpublished observations, personal communication) suggest that calcium sensitization may be occurring through activation of Rho kinase. Evidence from both of these laboratories has indicated that the Rho kinase inhibitor Y-27632 inhibits the effects of microtubule disruption on VSM contractile function. It is significant that Rho kinase function has been linked to focal contacts (20). Obviously, more work will be required to elucidate the mechanism(s) responsible for the effect of microtubules on contractile tone.

It is also possible that processes regulating the organization of the actin cytoskeleton may be essential in determining the role integrins play in mechanotransduction. Force generated by contractile proteins is transmitted directly to the dense plaque region of the cell. Likewise, application of external force to the cell is transmitted from the dense plaque region to the actin cytoskeleton. Cippola and Osol (5) reported that cytochalasin B, an inhibitor of actin polymerization, reduced the ability of cerebral arteries to tolerate increases in intravascular pressure, resulting in forced dilation. They speculated that the state of the actin cytoskeleton may be important for regulating responses to altered intravascular pressure. In airway smooth muscle, a large body of evidence is accumulating that suggests the actin cytoskeletal network is quite plastic in nature and capable of undergoing relatively rapid remodeling (19). Such processes may be particularly important in determining the smooth muscle responsiveness to mechanical forces.

**ROLE OF MAPKS IN MYOGENIC RESPONSE**

A fourth line of evidence for integrin involvement in the myogenic response is that downstream targets of integrin-ECM interactions alter Ca\textsuperscript{2+}-dependent signaling mechanisms in VSM. In particular, tyrosine phosphorylation events involving mitogen-activated protein kinases (MAPK) and Rho kinase have been implicated in modulation of Ca\textsuperscript{2+} sensitivity of the contractile apparatus (14, 46). It is important to note that these kinases exist in phosphorylation cascades and are anchored to CSK scaffolds linked to the extracellular matrix through integrins (20, 27).

The data supporting a possible role for MAPK in the regulation of VSM tone are controversial. To date, investigations have largely employed strategies involving inhibitors or activators. Nonspecific phosphotyrosine inhibitors cause concentration-dependent vasodilation in cannulated arterioles exhibiting spontaneous myogenic tone (61). However, those arterioles continue to demonstrate pressure-dependent vasoconstriction in the presence of the inhibitors, suggesting that constitutive tyrosine phosphorylation is not fundamental to the myogenic response but may be involved in parallel pathways that modulate basal arteriolar tone. Similar results (33, 62) have been obtained using the MAPK/extracellular signal-regulated protein kinase (MEK) inhibitor PD-98059. Also, the nonspecific tyrosine phosphatase inhibitor pervanadate causes vasoconstriction of cannulated, pressurized arterioles (45). In rat mesenteric arteries, a synergistic effect of angiotensin II and intraluminal pressure was demonstrated on the phoshorylation and activation of the p42/44 MAPKs (36). Spurrell et al. (62) also demonstrated increased phosphorylation of p42 MAPK in cannulated rat cremaster muscle arterioles following an acute pressure step (30–100 mmHg). Loufrani et al. (31) reported that isometric stretch of rabbit facial vein segments invokes two distinct signaling pathways: one related to generation of myogenic tone and the other to activation of p42/44 MAPK. The effect of stretch was specifically isolated from the generation of myogenic tone by examining MAPK kinase activation at 33°C, a temperature at which the facial vein behaves passively. In cannulated cremaster muscle arterioles, increased intraluminal pressure results in the accumulation of phosphotyrosine within the VSM, and this persists despite inactivation of the contractile elements by a number of mechanisms, including removal of extracellular Ca\textsuperscript{2+}, calcium channel blockade, or forskolin treatment (Murphy TV, Spurrell BP, and Hill MA, unpublished observations). Together these studies suggest that the mechanical stimulation provided by either tissue stretch or an increase in intraluminal pressure may activate both MAPK and contraction, but these responses may not be directly related. However, it remains conceivable that activation of MAPK potentiates or facilitates myogenic responsiveness through actions on Ca\textsuperscript{2+}-sensitive pathways and thin filament regulatory mechanisms.

In contrast to the possible involvement of p42/44 MAPK, there are no published data available regarding a specific role for p38 MAPK or cJun NH\textsubscript{2}-terminal kinase/stress-activated protein kinase (JNK/SAPK) in the arteriolar myogenic response. However, preliminary studies using the specific p38 inhibitor SB-203580 suggest that this particular kinase is not directly in-
involved in the myogenic contraction of cannulated rat cremaster muscle arterioles (62). Despite this, these enzymes are known to be present in contractile tissues and can be activated by mechanical stimuli (stretch), agonists (angiotensin II), and reactive oxygen species (17, 45, 76). Furthermore, it has been suggested that activation of p38 MAPK, through downstream phosphorylation of heat shock protein 27, is involved in modulation of arterial smooth muscle contraction following exposure to endothelin (76).

An important question in understanding the role of MAPKs in arteriolar smooth muscle mechanotransduction relates to signaling molecules lying upstream from activation of the kinase. Although MAPK activation has been implicated in a number of signaling pathways relevant to this review, significant evidence exists for an axis involving ECM, integrin binding, formation of focal adhesions, and activation of upstream kinase enzymes such as Src family tyrosine kinases (4, 13, 55). Consistent with this, studies using the PTK inhibitor herbimycin A suggest the possible involvement of c-Src in myogenic responsiveness of pressurized cerebral arteries (34, 35). Similarly, the activation of p42/44 MAPKs by angiotensin II in pressurized vessels was inhibited by herbimycin A (36). More recently, Murphy et al. (43) show that the Src inhibitor PP1 dilates pressurized rat cremaster muscle arterioles but does not prevent the accumulation of total phosphotyrosine residues that occurs in arteriolar smooth muscle following an increase in intraluminal pressure. Whereas further studies are required to delineate the role of MAPK activation in arteriolar mechnotransduction, it is conceivable that an increase in intraluminal pressure activates signal transduction pathways that are not directly involved in acute myogenic vasoconstriction. For example, increased wall tension may activate processes leading to compensatory changes in the structure of the vascular wall (such as hypertrophy and remodeling). It is also conceivable that myogenic vasoconstriction, through opposing an increase in wall tension, may remove or attenuate the stimulus for activation of signaling mechanisms involving MAPKs.

SUMMARY AND CONCLUSIONS

As clearly indicated, there is a paucity of direct evidence to support an obligatory role for integrins in the vascular myogenic response. The most compelling data are those showing inhibition of myogenic tone by integrin-specific peptides. Additionally, recent experiments show that integrins regulate the same VSM calcium entry pathway that is required for myogenic tone. These data suggest at least a modulatory role for integrins in the genesis of vascular myogenic tone. Supporting arguments for an ECM-integrin-CSK-PTK axis in pressure transduction can also be made based on the vascular effects of cytoskeletal protein disrupters and inhibitors of MAPK and Rho kinase pathways. Clearly, more direct tests for each element of this axis must be made using preparations in which the components of myogenic- and agonist-induced tone can be delineated.

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