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REVIEW ARTICLE

Regulation of Ion Channels by Integrins

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Abstract

Ion channels are regulated by protein phosphorylation and dephosphorylation of serine, threonine, and tyrosine residues. Evidence for regulation of channels by tyrosine phosphorylation comes primarily from investigations of the effects of growth factors, which act through receptor tyrosine kinases. The purpose of the present work is to summarize evidence for the regulation of ion channels by integrins, through their downstream, nonreceptor tyrosine kinases. We review both direct and indirect evidence for this regulation, with particular emphasis on Ca^{2+}-activated K^+ and voltage-gated Ca^{2+} channels. We then discuss the critical roles that cytoskeletal, focal-adhesion, and channel-associated scaffolding proteins may play in localizing nonreceptor tyrosine kinases to the vicinity of ion channels. We conclude by speculating on the physiological significance of these regulatory pathways.

Index Entries: Receptor tyrosine kinase; nonreceptor tyrosine kinase; integrins; cytoskeleton; focal adhesion; growth factor receptors; scaffolding proteins; Src; FAK; AKAP; SH3.

INTRODUCTION

Ion channels are the targets of many intracellular signaling pathways, including protein phosphorylation and dephosphorylation. Indeed, nearly every type of voltage-gated K^+, Ca^{2+}, and Na^+ channel is regulated to some extent by phosphorylation of serine/threonine residues on intracellular domains of the channel *(1,2)*. Phosphorylation can alter channel gating properties, including voltage sensitivity and calcium sensitivity, and thereby dramatically control the electrophysiological properties of a cell. In addition to serine/threonine phosphorylation, considerable recent evidence suggests that ion channels are also regulated by phosphorylation on tyrosine residues *(1,3–7)*.

Evidence for regulation of ion channels by tyrosine phosphorylation comes primarily from investigations of the effects of growth factors. Growth factors, which act through receptor protein tyrosine kinases (PTKs), regulate the long-term expression of ion channels *(8,9)* but

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also have acute actions on channel activity. Receptor PTKs are characterized by an extracellular, ligand-binding domain, a transmembrane domain, a kinase-catalytic domain and cytoplasmic regions responsible for coordinating the subsequent activity of signaling molecules. Signal transduction involves growth factor (ligand) binding to the extracellular domain, dimerization of the receptor proteins, and autophosphorylation of the receptor. Receptor autophosphorylation then creates phosphorylated tyrosine residues on the cytoplasmic tail of the receptor, which form docking sites for signaling molecules. The combination of these signaling molecules determines the specificity of individual receptor PTKs *(10).*

Nonreceptor PTKs can also regulate ion channels. These enzymes play a prominent role in signaling pathways downstream from integrins and other adhesion molecules. Nonreceptor PTKs are found in both the cytoplasm and nuclei of cells, but the largest family is the cytoplasmic Src (sarcoma virus tyrosine kinase) family *(11),* consisting of eight members, including Src, Fyn, and Yes, that are ubiquitously expressed. Regulation of Src family members is highly conserved: Autophosphorylation of a kinase domain tyrosine leads to increased kinase activity, whereas phosphorylation of a tyrosine residue near the C terminus represses activity *(12).* Many stimuli, including receptor PTKs, G-protein coupled receptors, and integrins have been implicated in Src activation, suggesting that this family of kinases is a key point of integration for many signal transduction pathways. Another relevant nonreceptor PTK is $pp125^{FAK}$, which is discretely localized to cellular focal adhesions and has been shown to colocalize with integrins. FAK is a substrate for integrin-dependent tyrosine phosphorylation and becomes enzymatically active upon phosphorylation, serving as a scaffold for the binding and localization of other proteins to the focal adhesion *(13).* Activation of Src family members and FAK serves as a key integration mechanism for a number of extracellular signaling pathways.

The regulation of ion channels by growth factors and receptor PTKs has been reviewed, to some extent, previously *(3,4,6,7).* The purpose of the present work is to summarize evidence for the regulation of ion channels by integrins and integrin-linked tyrosine kinases. We review both direct and indirect evidence for this regulation. We then discuss the critical roles that the cytoskeleton and channel-associated scaffolding proteins may play in localizing PTKs to the vicinity of ion channels. When possible, we speculate on the physiological significance of these regulatory pathways.

INTERACTIONS BETWEEN INTEGRINS AND ION CHANNELS

Integrins are a family of membrane-spanning glycoproteins that link the extracellular matrix (ECM) to the cytoskeleton. Integrins are composed of α–β heterodimers with extracellular domains that bind ECM proteins and short cytoplasmic tails that associate with focal adhesion proteins *(14,15).* As mentioned earlier, integrin activation is a well-known trigger of intracellular tyrosine phosphorylation cascades. Integrin engagement by multivalent ligands, including extracellular matrix proteins, induces receptor clustering, the recruitment of cytoskeletal proteins to the focal adhesion, and the activation of nonreceptor PTKs *(16,17).* Because integrins lack intrinsic enzymatic activity, they rely on the activation of other cytoplasmic signaling molecules, including FAK and Src. Integrin interaction with FAK leads to FAK autophosphorylation, to the creation of a binding site for the Src SH2 domain, and, ultimately, to Src activation *(18).* Src then phosphorylates additional sites on FAK to allow binding of other signaling molecules and scaffolding proteins *(19,20).* This process leads to the assembly of complex signaling molecules at the focal adhesion site and organizes further downstream signaling events *(15).* Many of the signaling pathways activated by receptor PTKs overlap with integrin-mediated signaling pathways, utilizing the same PTKs through different adaptor proteins *(21,22).*

Integrins may play a role in directing the localization of ion channels. In neuroblastoma

cells, neurite outgrowth is initiated by hyperpolarization subsequent to β1-integrin-dependent adhesion on extracellular matrix *(23).* G-protein-coupled inwardly rectifying K^+ (GIRK) channels are implicated in this hyperpolarization, although the mechanism and possible coupling of these channels to β1 integrins has not been elucidated. In oocytes, GIRK1 and GIRK4 channels coimmunoprecipitate with an endogenous β1-integrin subunit *(24).* The Asp-Gly-Arg (RGD) sequence is thought to be an integrin-specific recognition sequence contained in many extracellular matrix proteins and this sequence is also found in the first membrane-spanning (extracellular) region of all known GIRK channels, but not in any other cloned K^+ channels *(24).* Interestingly, mutation of the RGD site (to RGE) on the channel decreases or eliminates GIRK current after expression of the mutant channel. However, RGD peptides, applied extracellularly, do not modulate GIRK channel current. This evidence suggests that the RGD site is important for insertion of the channel into the cell membrane, rather than for acute regulation of the channel *(24).* However, only the aspartate residue, not the entire RGD motif, appears to be important for proper GIRK processing and function *(25).* Thus, the functional association between GIRK channels and β1 integrins remains uncertain at this time.

There are other examples of links between the expression of ion channels and integrins. HERG (ether-a-go-go-related gene) K^+ channels have recently been shown to play a role in regulation of integrin expression. Adherence of FLG 29.1 preosteoclasts on fibronectin (FN) activates HERG current and the activation is sustained by β1-integrin-subunit activation. The sustained activation of HERG current appears necessary for upregulation of αvβ3 integrin in these cells *(26).* The cystic fibrosis transmembrane regulator, a Cl^- channel, functions as an adhesion receptor for at least two types of bacteria *(27).* A member of a closely related family of Ca^{2+}-activated Cl^- channels, CLCA2, serves as a β4-integrin-binding partner for adhesion between endothelial cells and breast cancer cells *(27).*

In addition to a possible role in channel localization, evidence from a large number of studies indicates that integrins play a functional role in ion channel regulation. For example, integrin-dependent adhesion initiates Ca^{2+} influx in endothelial cells *(28,29),* fibroblasts *(30),* osteoclasts *(31–33),* leukocytes *(34),* hepatocytes *(35),* smooth-muscle cells *(36)* and epithelial cells *(37).* Integrins are involved in the mechanical modulation of neurotransmitter release *(38,39).* Integrin-dependent adhesion inititiates hyperpolarization in neurons *(23,40).* Integrin-specific peptides (RGD, LDV) cause vasodilation and vasoconstriction in isolated skeletal muscle arterioles *(41),* responses that are blocked by inhibitors of K^+ and Ca^{2+} channels, respectively *(42).* Integrin-mediated tyrosine phosphorylation cascades have been implicated in a number of processes that involve ion channels *(43).* The majority of these studies are summarized in Tables 1 and 2.

Relatively few studies have defined specific interactions between an integrin and a channel. The exceptions are noted in Table 1. For the sake of brevity, we will discuss only a few selected studies. K_{Ca} channels in erythroleukemia cells are activated following cell contact with fibronectin-coated microspheres, which are known to engage and aggregate several β1 and β3 integrins. Over a time-course of 800 s, there is an increase in whole cell K_{Ca} current and a 40-mV hyperpolarization *(40).* Although this evidence suggests possible signaling between β1 and/or β3 integrins and K_{Ca} channels, single-channel currents were also activated away from the pipet attachment site, leaving open the possibility that the channels were activated by global or localized increases in intracellular calcium rather than by direct association with integrins. A more recent study by the same group shows that HERG current in an osteoclast cell line is activated by adhesion on FN. The exact signaling pathway was not defined, but it involves a pertussis-toxin-sensitive G_i-protein *(26).*

More direct evidence for a functional association between channels and integrins exists for T-lymphocytes. In T-cells, $K_v1.3$ channels are

Table 1
Evidence for K^+ Channel Regulation by Integrins

Channel	Integrins	Effect on channel activity	Channel inhibitor	Integrin ligand	Evidence for protein phosphorylation	Notes	Preparation-cell type	Ref.
$K_v1.3$	β1	+?	MgTx, KaTx, NoTx, 4-AP, quinine	FN, anti-β1 Ab		Trigger T-cell β1 integrins function by opening $K_v1.3$; $K_v1.3$ and β1 integrins coimmunoprecipitate	Human T-cell	Levite et al. *(44)*
GIRK	β1			RGD		GIRK1 and GIRK4 channels coimmunoprecipitate with integrin subunit. RGD sequence in channel essential for activity	*Xenopus laevis* oocytes	McPhee et al. *(24)*
GIRK	β1			RGD		GIRK1 and GIRK4 channels do not require β1 integrins for expression and function	Fibroblast cell line	Ivanina et al. *(25)*
K_{ir}	VLA-4 (?), α4β1 (?), α4β7 (?)	–		VCAM-1		Monocyte adherence to VCAM produces hyperpolarization sufficient to promote monocyte Ca^{2+} entry	Human THP-1 monocytes	Colden-Stanfield and Scanlon *(45)*

K_{ir}	VLA-4 (?), α4β1 (?), α4β7 (?)	+	Ba^{2+}	VCAM-1, anti-VCAM-1 Ab	Herbimycin A	Adhesion of monocytes to LPS-treated HUVECs or VCAM-1 increases the expression of Kir channels	Human THP-1 monocytes	Colden-Stanfield and Gallin *(46)*
K_{ir}	β1	+	Ba^{2+}, Cs^{+}	FN, VN, RGD	Pertuxis toxin, FAK, herbimycin A	Neuritogenesis depends on FN-induced hyper-polarization caused by activation of K_{ir}; G-protein, and PTP involved	N1 subclones of the 41A3 neuro-blastoma cells	Arcangeli et al. *(23)* Bianchi et al. *(47)*
K_{Ca}	α5β1	+		RGD, anti-integrin Abs	Genistein, neomycin, flu-narizine, W7, H7, calphostin C, cytocha-lasin D	Activation of apamin-sensitive, K_{Ca} channels; hyperpolarization at 0.33 Hz strains; actin cytoskeleton, PLC-calmodulin, PTK, and PKC are involved	Human articu-lar cartilage chondrocytes	Wright et al. *(48)*
K_{Ca}	CD47, α5, β1	+	Gd^{3+}, quini-dine, apamin	Integrin Abs, RGD	Genistein	Activation of small conductance K_{Ca} channels; cyclical stain at 0.33. Cytochalasin D inhibited hyper-polarization.	Human bone cells	Salter et al. *(49)*

(continued)

Table 1
(Continued)

Channel	Integrins	Effect on channel activity	Channel inhibitor	Integrin ligand	Evidence for protein phosphorylation	Notes	Preparation-cell type	Ref.
K_{Ca}	α5β1	+	Apamin, Gd^{3+}	Anti-β1 Ab	Genistein, neomycin, flunarizine	Mechanical strain-α5β1: (1) activation of SAC and actin cytoskeleton, (2) release of IL-4, activating PLC, IP_3-mediated Ca^{2+} release and apamin-sensitive K_{Ca} channels	Human articular chondrocytes, IL-4 knockout mice	Millward-Sadler et al. *(50)*
K_{Ca}	β1 (?), β3 (?)	+	TEA	FN, RGD		Hyperpolarization	Murine erythroleukemia cells	Arcangeli et al. *(51)* Becchett et al. *(40)*
HERG K^+	β1	+		FN		HERG K^+ activation by FN required for αvβ3 expression	Human leukemic pre-osteoclastic cell line (FLG 29.1 cells)	Hofmann et al. *(26)*
K^+	αvβ3 (?)	+	TEA, 4-AP, glibenclamide, Ba^{2+}	RGD		K_{ir}, K_v and K_{ATP} blockers partially inhibit RGD vasodilation	Rat cremaster arterioles	Platts et al. *(41)*

Abbreviations: 4-AP, 4-aminopyridine; MgTx, margatoxin; KaTx, kaliotoxin; NoTx, noxioustoxin; FN, fibronectin; VN, vitronectin; GIRK, G-protein-activated inward rectifier K^+ channels; ChTX, charybdotoxin; VCAM-1, vascular cell adhesion molecule-1; HERG K^+ channels, encoded by the ether-a-go-go-related (herg); RGD, Arg-Gly-Asp peptide; K_v, voltage-gated K^+ channel; K_{ir}, inward rectifier K^+ channel; K_{ATP}, ATP-sensitive K^+ channel; K_{Ca}, Ca^{2+}-activated K^+ channel; PLC phospholipase C; PKC, protein kinase C; Ab, antibody; LPS, lipopolysaccharide.

Table 2
Evidence for Ca^{2+} and Na^{+} Channel Regulation by Integrins

Channel	Integrins	Effect on channel activity	Channel inhibitor	Integrin ligand	Evidence for protein phosphorylation	Notes	Preparation-cell type	Ref.
Na^{+}	αv, β1, β5	+	TTX	Integrin Ab, RGD	Genistein, cytochalasin D	Cyclical stain depolarizes; blocked by integrin Ab's	Human bone cells	Salter et al. *(49)*
Na^{+} or NSC	α5β1	+	TTX, Gd^{3+}	Anti-α5, anti-β1 Ab	Genistein, neomycin	Depolarization at 0.33 Hz strain; PLC and PTK are involved	Osteoarthritic articular cartilage chondrocytes	Millward-Sadler et al. *(52)*
SAC (NSC?)	α5β1	+				Hyperpolerization to mechanical stimulation is mediated by paracrine factor; blocked by IL-4 Ab	Human articular chondrocytes	Millward-Sadler et al. *(53)*
SAC (NSC?)	α5β1		Gd^{3+}	Oligopeptides (containing RGD)	FAK, β-catenin, paxillin	Tyrosine phosphorylation of FAK, paxillin, β-catenin through α5β1 requires SAC activity	Human articular chondrocytes	Lee et al. *(54)*
SOC?	β1, αv	+	Ca^{2+}-free bath	FN, VN, anti-αvβ3 Ab		Adhesion-dependent $[Ca^{2+}]_i$ increase	Human umbilical vein endothelium	Schwartz *(55)* Schwartz and Denninghoff *(28)*
SOC?	αIIbβ3	+		GPIIb-IIIa Ab, RGD		GPIIb–IIIa is involved in Ca^{2+} channel activation	Platelet plasma membrane	Fujimoto et al. *(56)*

(continued)

Table 2
(Continued)

Channel	Integrins	Effect on channel activity	Channel inhibitor	Integrin ligand	Evidence for protein phosphorylation	Notes	Preparation-cell type	Ref.
SOC?	αvβ3, αvβ5	+	Ni^{2+}	RGD		RGD peptides on beads induce Ca^{2+} entry; this Ca^{2+} signal promotes adhesion	Madin–Darby canine kidney epithelial cells	Sjaastad et al. *(57)* Sjaastad et al. *(37)*
SOC?	αIIbβ3	+		αIIbβ3 Ab, RGD peptide		VWF binding to GPIb is responsible for αIIbβ3-dependent Ca^{2+} influx, αIIbβ3 complex may function as Ca^{2+} channel	Platelet	Bertolino et al. *(58)*
SOC?	αvβ3	+	Ca^{2+}-free bath, thapsigargin	LM609, VN	Genistein	$[Ca^{2+}]_i$ increase evoked by αvβ3 clustering; 70% Ca^{2+} entry; 30% Ca^{2+} release	Bovine pulmonary artery endothelium	Bhattacharya et al. *(29)*
CaL	αvβ3	–	Nifedipine	FN, VN, anti-β3 Abs, RGD		VN and αvβ3–Ab inhibit current; FN potentiates current	Rat cremaster artetriolar smooth muscle	Wu et al. *(59)*
CaL	α5β1	+	Nifedipine	FN, anti-α5 Ab	FAK–Ab, Src–Ab, genistein, piceatannol	Potentiation of current by α5β1; blocked by PP2, Src SH2 inhibitor, FRNK, paxillin Ab, vinculin Ab, peptides for PTP site on channel C terminus	Rat cremaster arteriolar smooth muscle	Wu et al. *(59)* Wu et al. *(60)*

CaL	α4β1	+	Nifedipine	LDV, anti-α4 Ab	PP2, calphostin C	LDV peptide and α4β1 potentiate current and constrict	Rat cremaster arteriolar	Waitkus-Edwards et al. *(42)*
CaL	α7	+	Cd^{2+}, nifedipine	Anti-α7, Ab, laminin		KXGFFKR motif in α7 integrins modulates Ca^{2+} signal; calreticulin couples Ca^{2+} release and influx; exocalreticulin elicits Ca^{2+} influx	E63 Skeletal muscle cells	Kwon et al. *(61)*
CaL	β1		β-adrenergic receptor agents	Anti-β1 Ab, laminin	Cytochalasin D	Laminin–β1 integrin–actin cytoskeleton interaction reduces β1-adrenergic and enhances β2-adrenergic modulation of the channel	Cat atrial myocytes	Wang et al. *(62)*
CaL	β1	–	ACh	Anti-β1, laminin, YIGSR peptide	Spermine–NO, milrinone, IBMX, forskolin, cytochalasin D	Laminin–β1 integrin–actin cytoskeleton interaction inhibits adenylate cyclase and ihibits NO-mediated increase of channel after ACh withdrawal	Cat atrial myocytes	Wang et al. *(63)*
CaL-N	β1, β3?	+	Cd^{2+}	FN		FN fragment and NGF potentiate HVA Ca^{2+} current	Molluscan neurons	Wildering et al. *(64)*

Abbreviations: SAC, stretch-activated channels; NSC, nonselective cation channel; FN, fibronectin; VN, vitronectin; PTK, protein tyrosine kinases; ChTX, charybdotoxin; VCAM-1, vascular cell adhesion molecule-1; SOC, store-operated Ca^{2+} channels; PAO, phosphatase inhibitor phenylarsine oxide; FRNK, a C-terminal, noncatalytic domain of FAK lacking tyrosine kinase activity; PTP, protein tyrosine phosphorylation; RGD, Arg-Gly-Asp peptide; Ab, antibody.

necessary for activation of β1 integrins and subsequent integrin-dependent adhesion and migration *(44).* This mechanism underlies the activation of T-cell adhesion by elevation of extracellular K^+ in the absence of any specific receptor-mediated event and accounts for the ability of substance P, which inhibits $K_v1.3$ channels, to inhibit T-cell adhesion. Although it has not yet been tested whether the signaling between $K_v1.3$ channels and β1 integrins works in reverse, $K_v1.3$ and β1 integrins coimmunoprecipitate, suggesting that direct physical association of these molecules underlies their functional interaction *(44).*

Several lines of evidence suggest that calcium channels are acutely regulated by integrin-dependent signaling. Most of this evidence is summarized in Table 2. In many cell types, integrin engagement triggers both intracellular Ca^{2+} release and Ca^{2+} influx across the plasma membrane *(65),* and these two processes are often coupled. This phenomenon can be illustrated by a discussion of endothelial cell–integrin interactions. Integrin-dependent adhesion initiates Ca^{2+} influx in endothelial cells *(28).* Application of the ECM protein, vitronectin, or a polyclonal antibody to αvβ3 integrin stimulates endothelial cell Ca^{2+} release and Ca^{2+} influx *(29).* These ligands also stimulate tyrosine phosphorylation of multiple endothelial cell proteins *(66).* Both Ca^{2+} influx and protein phosphorylation are blocked by soluble tyrosine kinase inhibitors *(29).* The ion channel responsible for Ca^{2+} influx has not been identified, but it is likely to be the so-called "store-operated Ca^{2+} influx channel." The current most likely to be associated with this process is a small inward, Ca^{2+}-selective current in nonexcitable cells, termed I_{crac}, for Ca^{2+}-release-activated Ca^{2+} current *(67).* Molecularly, the best candidates for I_{crac} are the *Trp* (transient receptor potential) family of proteins *(68)* and the channellike intestinal calcium transporter CaT1 *(69,70).* Recombinant *Trp* channels share many, but not all, characteristics of I_{crac} *(71,72),* although heterologous combinations of *Trp* isoforms in native cells may account for the unique characteristics of some endogenous store-operated currents *(73,74).* A recent study shows that heterologously expressed CaT1 protein is nearly indistinguishable from I_{crac} *(70).* Of relevance to the possible regulation of store-operated Ca^{2+} current by integrins is the recent finding that αvβ3 ligands activate store-operated Ca^{2+} current in human umbilical vein endothelial cells *(75).* Additional evidence indicates that I_{crac} can be regulated by tyrosine kinases *(76–79)* and that its full activation requires cytoskeletal proteins *(80,81)* and small GTPases *(82).*

In contrast to the circumstantial evidence for regulation of Ca^{2+} current in nonexcitable cells by integrins, the evidence for regulation of voltage-gated calcium channels is much stronger. In vascular smooth muscle, the L-type calcium channel is regulated by at least three different integrins. Whole-cell recordings from vascular myocytes show that soluble ligands of the αvβ3 integrin, such as RGD peptides, vitronectin, and fibronectin fragments, inhibit L-type current *(59).* Interestingly, insoluble αvβ3 ligands (i.e., ligands attached to microspheres) also inhibit current *(59).* This is somewhat counter to what might be expected, because soluble (monovalent, nonclustering) integrin ligands do not trigger the entire ensemble of downstream signaling events (i.e., cytoskeletal protein redistribution and tyrosine phosphorylation) typical of integrin–multivalent ligand interaction *(83).* Rather, monovalent integrin ligands are thought to act by disrupting existing interactions between integrins and their insoluble ligands. Because both soluble and insoluble αvβ3 integrin ligands produce the same effects on smooth-muscle-cell calcium current, both may be triggering the same signaling pathway involving Ca^{2+} channels. The signaling pathway by which αvβ3 integrin regulates the L-type channel is not known, but it appears to be insensitive to tyrosine kinase inhibitors (Wu, unpublished observations). In contrast to inhibition of Ca^{2+} current by αvβ3 ligands, soluble ligands (LDV peptide) of the laminin receptor, α4β1, enhance L-type calcium current *(84).* However, the α4 integrin antibody does not modulate current, although pretreat-

ment with the antibody blocks the LDV response. These observations suggest that the signaling pathways between these two integrins and the L-type Ca^{2+} channel in this tissue are fundamentally different. Whether either signaling pathway involves changes in the phosphorylation state of the channel is not known.

Substantially more information is available about how insoluble α5β1 integrin ligands modulate the L-type calcium channel. L-Type calcium current in vascular smooth muscle is potentiated up to twofold by insoluble α5β1 ligands, and this potentiation is blocked by soluble tyrosine kinase inhibitors, including genistein, piceatannol, and the Src-family-specific inhibitor, PP2. Tyrosine phosphatase inhibition increases basal L-type calcium current *(60,85).* Although it might be argued that these inhibitors lack specificity, cell dialysis with antibodies to c-Src or focal adhesion kinase (FAK) also block potentiation of current, with a lesser but significant effect on basal current. Recent experiments demonstrate many of the same effects of α5β1 ligands in heterologously expressed L-type Ca^{2+} channels (Gui et al., unpublished observations), indicating that these conclusions do not only apply to freshly dissociated smooth-muscle cells, which could potentially be altered by enzyme treatment. These observations also fit with data from other types of smooth muscle where Ca^{2+} current is increased by intracellular application of constitutively active Src kinase *(86),* increased by c-Src activating peptide *(87),* and inhibited by a c-Src monoclonal antibody *(88).*

Similarities between the actions of integrin ligands and the growth factor PDGF (platelet-derived growth factor) on the L-type Ca^{2+} channel also support a role for regulation of the channel by tyrosine phosphorylation. PDGF, which stimulates tyrosine phosphorylation of multiple smooth-muscle proteins, enhances L-type calcium current *(88,89)* and increases tyrosine phosphorylation of the pore-forming channel subunit α_{1C} ($Ca_v1.2b$). In PDGF-stimulated smooth muscle, α_{1C} coimmunoprecipitates with c-Src *(88).* c-Src also interacts with the neuronal isoform of the α_1 Ca^{2+} channel subunit. In cerebellar granule neurons, α_{1C} is tyrosine phosphorylated in response to the insulin-like growth factor-1 (IGF-1), resulting in potentiation of L-type current *(90,91).* This effect can be duplicated with recombinant α_{1C} ($Ca_v1.2c$) expressed in SH-SY5Y cells. c-Src mediates this response because expression of kinase-dead Src, or application of PP2, blocks potentiation of current *(92).* Kinase assays using lysates from neuroblastoma cells expressing α_{1C} show that purified Src kinase phosphorylates Y^{2122} on the α_{1C} C terminus. Furthermore, point mutation of $Y^{2122}F$ prevents tyrosine phosphorylation and IGF-1 potentiation of current *(92).* Because $Ca_v1.2b$ and $Ca_v1.2c$ share identical sequences in this region, it is likely that Y^{2122} also mediates potentiation of current in smooth muscle by both PDGF and integrin ligands. In fact, a peptide designed to compete with phosphorylation of this region of the channel prevents potentiation of current in vascular smooth muscle by α5β1 integrin ligands *(60)* and in $Ca_v1.2b$ channels expressed in HEK 293 cells (Gui et al., unpublished observations).

In smooth-muscle cells, intracellular dialysis with antibodies to two integrin-associated cytoskeletal proteins, paxillin and vinculin, also blocks regulation of L-type current by α5β1 ligands. The ability of vinculin and paxillin antibodies to do this is likely the result of their interference with the assembly of Src or another nonreceptor PTK on an intracellular scaffold of focal adhesion proteins, rather than a direct interaction with the channel. Src homology 2 (SH2) and Src homology 3 (SH3) domains in these proteins enable them to associate with PTKs *(14).* As mentioned earlier, the C termini of $Ca_v1.2b$ and $Ca_v1.2c$ contain at least two proline-rich domains that may interact with SH3 domains in Src, Lyn, and Hck tyrosine kinases *(93)* and possibly with SH3 domains in docking, adaptor, or scaffolding proteins. Deletion of one proline-rich region results in increased channel current, suggesting that it is constitutively involved in channel inhibition *(94).* However, it is not yet known whether phosphorylation

of the α_{1C} C terminus by any of these PTKs alters this inhibitory property.

Collectively, these observations suggest that L-type Ca^{2+} channels are regulated by multiple intracellular pathways downstream from αvβ3, α5β1, and α4β1 integrins. What is the physiological relevance of these observations? We propose that the extracellular matrix exerts constitutive control over the major calcium-permeable ion channel in vascular smooth muscle and that acute regulation of calcium signaling by integrins may occur under physiological and pathological conditions in blood vessels. This conclusion is consistent with evidence that integrin ligands and ECM proteins can acutely regulate vascular tone. For example, peptides containing the integrin-specific RGD (Arg-Gly-Asp) and LDV (Leu-Asp-Val) amino acid sequences cause dilation and constriction, respectively, of isolated, pressurized skeletal muscle arterioles *(42,95).* The dilation to RGD peptides is associated with a fall in intracellular calcium *(96)* and is blocked by a β3-integrin antibody *(95).* In contrast, α5β1- and α4β1-integrin ligands produce constriction of the same arterioles. This evidence suggests that extracellular matrix proteins have the potential to influence vascular tone through an interaction with integrins, pointing to a possible role for these mechanisms in tissue injury responses *(97).* Whether this occurs in other cell types is not known, but it is interesting to note that the ECM protein, FN, and the nerve growth factor (NGF) acutely enhance high-voltage-activated Ca^{2+} currents in molluscan neurons *(64).* FN also enhances the frequency of action potential firing (W. Wildering, *see* Note Added in Proof) in that tissue, suggesting that a matrix–integrin–calcium channel signaling axis may acutely regulate electrical excitability of those cells.

Another extracellular matrix protein, laminin, modulates L-type Ca^{2+} current in atrial myocytes by a different mechanism. Interactions between laminin and β1 integrins reduces β1-adrenergic modulation of L-type Ca^{2+} current but enhances β2-adrenergic modulation of current *(62).* Additionally, the laminin–β1 integrin interaction inhibits adenylate cyclase and thereby alters L-type Ca^{2+} current *(63).* The signaling pathways involved in these actions of laminin remain to be elucidated; however, both effects require an intact actin cytoskeleton *(62,63).* Muscarinic inhibition of L-type Ca^{2+} current, and muscarinic activation of K^+ current, is absent in β1-integrin$^{-/-}$ cardiomyocytes *(98).* Interestingly, β-adrenergic modulation of the Ca^{2+} current is unaffected in this knockout *(98),* an observation that conflicts somewhat with the above reports *(62,63).* It is possible that this mode of regulation in cardiomyocytes compensates for lack of direct tyrosine phosphorylation by Src of the cardiac L-type Ca^{2+} channel ($Ca_v1.2a$), which has a substantially different C terminus and does not contain the critical tyrosine residue required for $Ca_v1.2b$ and $Ca_v1.2c$ potentiation *(92).*

Other adhesion proteins are also known to interact with ion channels. For example, the adhesion molecule PECAM, is constituitively expressed on endothelial cells, and a PECAM antibody evokes $[Ca^{2+}]_i$ increases in HUVECs *(99)* through activation of a nonselective cation current. Activation of this current requires an intact PECAM-1 cytoplasmic domain and Src kinase activity *(100).* Tenascin-C is an extracellular adhesive glycoprotein composed of a series of epidermal growth factor (EGF)-like repeats, a fibrinogen-like region, and a series of fibronectin-like regions *(101).* Neuronal Na^+ channels bind to tenascin-C with high affinity as well as to the related protein, tenascin-R, which lacks several of the fibronectin repeats *(102).* The Na^+ channel β2-subunit contains an Ig domain with close sequence similarity to the neural cell adhesion molecule contactin/F3 *(103).* Contactin/F3 binds tenascin and related ECM proteins. Additionally, the EGF-like domains of tenascin-R have the potential to regulate Na^+ channels through interactions with PTKs. Although a functional role for these extracellular proteins in the regulation of the Na^+ channel has not been demonstrated, it has been speculated that secretion of the proteins by neuronal support cells, such as oligodendrocytes, may direct localization of Na^+ channels on the corresponding neuronal cell membrane *(102).*

THE CHANNEL-PROTEIN KINASE REGULATORY COMPLEX

An emerging concept in the field of ion channel regulation is that modulation of channel function by phosphorylation requires the formation of a multiprotein complex. The pore-forming α-subunits of many channels bind to auxilliary channel subunits, but they also associate with scaffolding proteins that play essential roles in channel localization and activity *(3).* Scaffolding proteins link signaling enzymes, substrates, and potential effectors (such as channels) into a multiprotein signaling complex that may be anchored to the cytoskeleton. In addition to an obvious role in targeting the channel to a particular location on the cell membrane, there are at least three advantages to having an ion channel in a multiprotein complex. First, there is a large increase in efficiency of the kinetic reaction when an enzyme is localized with its substrate and effector in a microenvironment with restricted diffusion *(104).* Second, the anchoring of an enzyme complex to a channel may be necessary for the extremely rapid transmission of signals required to regulate some channels *(105).* Third, compartmentalization may be essential for determining specificity in signal transduction pathways *(106).*

There appear to be many families of scaffolding and adaptor proteins that potentially could be involved in organizing ion channels into signaling complexes and regulating function by coupling those channels to protein kinases. For serine–threonine kinases, prominent families are the MAGUK (membrane-associated guanylate kinase), AKAP (A-kinase associated) proteins, and GKAP (G-kinase associated) proteins *(107).* INAD (inactivation-no-after potential D) is another example of a multiprotein signaling complex. INAD is a *Drosophila* protein containing five PDZ domains (PSD-95/SAPSO, Dlg and 20-1 domains) that link together most of the proteins involved directly in phototransduction. This multiprotein complex includes rhodopsin, calmodulin, the putative store-operated Ca^{2+} channels *Trp* and *Trpl,* and the protein kinases PLC and PKC *(105,108).* INAD may serve as a template for understanding how other channels are regulated in a multiprotein complex.

Evidence that scaffolding proteins can mediate kinase-channel interactions is perhaps best established for the AKAP family of proteins *(109).* AKAPs serve to localize both kinases and phosphatases to multiprotein effector complexes that include K^+ and Ca^{2+} channels *(110).* A conserved AKAP anchoring motif directs dimerized PKA subunits to a particular subcellular target *(111).* For example, forskolin and cAMP potentiate ROMK1 (a Kir1.x subfamily of channels expressed in epithelial cells) current in native renal secretory cells *(112).* This potentiation is lost when ROMK1 is expressed in oocytes but restored if AKAP79 is coexpressed with the channel *(112).* AKAP15/18 is required for protein kinase A (PKA) potentiation of L-type Ca^{2+} channels in cardiac *(113),* skeletal *(114),* and vascular smooth muscle *(115).* AKAPs have also been implicated in the regulation of K_{Ca} channels *(116)* and the cystic fibrosis transmembrane conductance regulator (CFTR) *(117).* AKAP79 associates with the β2-adrenergic receptor and with MAGUK proteins, which, in turn, are coupled to glutamate-gated ion channels. AKAP79 has the potential to form part of a scaffold upon which PKA and PP2B (protein phosphatase 2B) may dually regulate the coupling of β2-adrenergic receptors and glutamate channels *(110).* Thus, AKAPs appear to be capable of assembling signaling complexes by virtue of their associations with ion channels and other scaffolding proteins. It is possible that AKAPs represent a general scheme for kinase regulation of channels and that similar families of adaptor proteins associated with other kinases, perhaps PTKs, will soon be identified.

ROLE OF THE CYTOSKELETON IN REGULATION OF ION CHANNELS

The cytoskeleton provides a backbone upon which scaffolding proteins are organized and thereby positioned to link kinases to ion chan-

nels. The cytoskeleton is a highly structured, three-dimensional network composed of three main components: actin filaments, microtubules (containing tubulin), and intermediate filaments (containing vimentin, neurofilament, etc.). Each component has a unique functional and structural role in the cell and the syncitial nature of the cytoskeletal network implies the existence of complex interrelationships among the various components. A large number of accessory proteins are involved in assembly, disassembly, and crosslinking of each of these elements. The prevailing view of the cytoskeleton, the tensegrity model *(118),* proposes a syncitium of compression-resistant struts (microtubules) suspended between various elastic elements (actin and intermediate filaments). Other structural proteins, including ezrin, ankyrin, spectrin, filamin, α-actinin, and talin, are required for anchoring the cytoskeleton into the plasma membrane and/or tethering it to other plasma membrane proteins.

There is increasing evidence to support a critical role for the cytoskeleton in the regulation of ion channels. One line of evidence is that cytoskeletal proteins are directly associated with ion channels. For example, the cGMP-gated cation channel of rod photoreceptors associates with spectrin *(119).* Accessory proteins associated with the *N*-methyl-D-aspartate (NMDA) receptor attach to the cytoskeleton (e.g., SAP97 binds band 4.1, an actin, and spectrin-binding protein *[120]).* The epithelial Na^+ channel associates with spectrin *(121).* NR1 and NR2 subunits of the NMDA receptor/channel bind to soluble tubulin and α-actinin *(122,123).* The list of these interactions is extensive and the reader is referred to several recent reviews for more detail *(3,111,124–126).* A second line of evidence is that cytoskeletal proteins are involved in various aspects of ion channel function. For example, the $K_v4.2$ channel interacts with the actin-binding protein filamin in cerebellar and hippocampal neurons *(127),* such that the magnitude of current is much greater when filamin is coexpressed with the channel than when the channel is expressed alone *(127).* At least one β-subunit of the $K_v1.1$ channel confers fast inactivation to the channel's α-subunit *(128,129)* and this property depends on the interaction of the subunits with F-actin and the phosphorylation state of the α-subunit *(130).* For $K_v1.5$ channels, disruption of the actin network using cytochalasin D or antisense constructs to α-actinin-2 result in increased $K_v1.5$ current, either by controlling channel gating or expression *(131).* Disruption of actin filaments in retinal bipolar cells also leads to activation of voltage-gated K^+ current *(132).* Collectively, these observations suggest that the actin cytoskeleton exerts a tonic, inhibitory effect on at least several types of K^+ channels.

The actin network appears to exhibit a different effect on Na^+ and Ca^{2+} channels. Actin filament disruption with cytochalasin D inhibits L-type Ca^{2+} current in vascular smooth muscle *(133)* and alters the time-course of activation of the cardiac Na^+ channel *(134).* The net effect of actin filament disruption on Na^+ and Ca^{2+} currents is to enhance current and alter the shape of the cardiac cell Ca^{2+} transient *(135).* Antibodies to F-actin are reported to alter the gating kinetics of the cardiac Na^+ channel by inducing a second open state and causing prolonged opening bursts *(134).* Fast inactivation of the Na^+ channel has been proposed to be controlled by a triplet of amino acid residues at the cytoplasmic II–IV linker region *(136).* Whether this region of the Na^+ channel is directly associated with cytoskeletal or scaffolding proteins is not yet clear.

An indirect way in which the actin network can influence ion channel gating is by controlling the translocation of protein kinases *(133).* Actin and vinculin are involved in translocation of c-Src *(137)* in response to growth factors or thrombin *(138,139).* Because Src has been shown to phosphorylate K^+ *(140–143)* and Ca^{2+} channels *(60,88,92),* disruption of actin filaments could alter the constitutive phosphorylation of those channels by Src or other Src-family kinases.

In addition to actin, other cytoskeletal proteins are implicated in the regulation of ion channels. Ankyrin is a large intracellular attach-

ment protein involved in connecting spectrin and actin to the cell membrane. Ankryin and spectrin associate with voltage-gated Na^+ channels in neurons *(144).* Ankyrin is required for clustering of Na^+ channels at nodes of Ranvier *(145),* axon hillocks *(146),* and in retinal ganglia *(147).* Cerebellum-specific knockout of $ankyrin_G$ results in an increased threshold for action potential firing in cerebellar neurons *(146).* Mouse cardiac myocytes lacking $ankyrin_B$ show reduced Na^+ channel current density and exhibit a variety of alterations in the function of the remaining cardiac Na^+ channels, most notably slowed recovery from inactivation *(148).* The defect resembles, in some respects, the action of selective Na^+ channel blockers *(149).* Intracellular dialysis with antibodies to β-spectrin or ankyrin is reported to alter gating kinetics of the cardiac Na^+ channel *(134).* Therefore, coupling of the Na^+ channel to ankyrin appears to be required for normal localization and function of this channel in heart and brain.

Ezrin–radixin–moesin (ERM) proteins are thought to serve as regulatable scaffolds that anchor actin filaments to the plasma membrane *(150).* Ezrin is identical to the 78-kDa AKAP that regulates type-II A-kinase in gastric parietal cells *(151).* Ezrin has also been identified as the AKAP that links PKA II to the CFTR chloride channel in secretory cells *(117).* It is noteworthy that ezrin is a well-known target of protein phosphorylation: It contains a PIP_2 binding site *(152)* and is a substrate for EGFR tyrosine kinase *(153).*

The ERM proteins also appear to be essential for both Rho- and Rac-induced cytoskeletal effects *(154).* Rho, Rac and Cdc42 belong to a family of low-molecular-weight GTPases that interact with ERM proteins and play essential roles in organizing the actin cytoskeleton. One downstream target of Rho, p^{160}ROCK, is known to phosphorylate myosin light-chain kinase and phosphatase to regulate assembly of actin–myosin filament bundles *(155).* This process is critical for reorganization of focal adhesions and adhesion-molecule clustering. These small GTPases are therefore downstream targets of integrin and growth factor signaling pathways *(156)* and are implicated as mediators of inside-out integrin signaling *(157).* Over a dozen target proteins have been identified for Rac and Cdc42 *(150),* including ion channels. For example, Rac 1 and Cdc42 are involved in the regulation of voltage-gated Ca^{2+} current by bradykinin *(158)* and in the regulation of I_{crac} *(82).*

Other downstream products of integrin and Ras-MAPK (mitogen-activated protein kinase) signaling *(159)* are also known to modulate ion channels. In cortical neurons, the L-type calcium channel is phosphorylated in response to β-amyloid, which accumulates extracellularly *(160).* This process is not sensitive to serine–threonine kinase inhibitors but is attenuated by PD98059, an inhibitor of MAPK *(160).* The use of antisense oligonucleotides to modify MAPK expression also reduces β-amyloid-induced Ca^{2+} accumulation, presumably through the L-type Ca^{2+} channel *(160).* Similarly, another small GTP-binding protein, Ras, mediates enhancement of mesangial cell Ca^{2+} current by Src and PDGF *(161).* This effect is specific for Ras, but not Rho or Rap 1. Several other studies support a role for Ras in the regulation of ion channels. Injection of H-Ras oncogenes into neurons enhances calcium currents *(162–164),* transfection of AtT-20 cells with Ras alters K^+ channel current, and tetrodotoxin (TTX) sensitivity of Na^+ channels *(164,166),* $p21^{ras}$ inhibits coupling of muscarinic receptors to inwardly rectifying K^+ (K_{ir}) channels in atrial cells *(167–169),* and Ras mediates acute inhibition of PC12 cell Na^+ channels by growth factors *(170).* Ras is necessary for the assembly of a signaling complex involving a mesangial cell Ca^{2+} channel, the PDGF-β receptor, and the adaptor proteins Grb2 and SOS (Son-of-sevenless) *(161).* Ras may mediate the enhancement of voltage-gated Ca^{2+} current by NGF and Src kinase in dorsal root ganglion neurons *(171).* Future studies are needed to elucidate how these small GTP-binding proteins interact with PTKs, protein tyrosine phosphatases (PTPs), and their putative multiprotein signaling complexes that regulate ion channels.

The other major component of the cytoskeleton, the microtubule system, has also been implicated in the regulation of ion channels. Colchicine, a microtubule disrupter, decreases the inactivation time constant of the cardiac L-type channel, thereby increasing the probability that the channel is in its closed state *(172).* The microtubule stabilizer taxol shifts the activation of cardiac Na^+ channels in such a way as to decrease the threshold for channel activation, which would potentially produce premature cardiac contractions *(134).* Microtubules appear to be involved in the inactivation of Ca^{2+} currents in snail neurons *(173)* but not in cardiac myocytes *(133,174).* Because microtubules are thought to represent compression-resistant struts that counteroppose contractile forces directed through the actin filament network *(175),* it is therefore likely that their disruption could result in secondary rearrangement of actin filaments and associated actin-binding proteins.

It should be noted that the specificity of cytoskeleton-disrupting agents must be considered when interpreting electrophysiological studies in which cytochalasin, colchicine, and so forth have been used. For example, low doses of colchicine that do not disrupt microtubules nevertheless inhibit the L-type Ca^{2+} channel in cardiac myocytes (albeit with less inhibition than at higher doses), implying that this agent works by an additional mechanism, perhaps by a direct block of the channel *(133).* Colchicine is known to competitively antagonize glycine receptors *(176).* Actin filament and microtubule disruption also produce a wide variety of effects on cell function that are unrelated to ion channels. For example, colchicine stimulates PKA activity by disrupting microtubules *(177),* which would indirectly alter the phosphorylation state of several types of channels. Many studies of the role of actin and tubulin in the regulation of particular channels do not appear to have addressed this issue. Therefore, elucidation of a role for specific cytoskeletal elements in the regulation of ion channels awaits the development of more selective tools to alter the function of these cytoskeletal proteins.

In summary, the regulation of ion channels by integrins most likely requires multiple cytoskeletal and focal adhesion proteins. The focal adhesion represents an insertion point for actin stress fibers in the cell membrane and an intracellular scaffold for the assemblage of many signaling and cytoskeletal components *(14).* Following integrin-dependent adhesion, kinases such as FAK, Src, phospholipase C (PLC)-γ, and Rho GTPase, as well as adaptor proteins such as Grb2, Sos, and Shc, are recruited to the focal adhesion underneath the ECM–integrin binding site *(14,156).* The process of recruitment requires, at a minimum, several cytoskeletal proteins and the small G-proteins that assemble them. Focal adhesions also contain a number of proteins such as paxillin, α-actinin, vinculin, talin, Cas, Crk, and so forth that are necessary for the association and regulation of PTKs and their targets. Although it is speculative at this time, it is possible to draw many parallels between the function of the focal adhesion and the function of channel–protein kinase complexes documented earlier in other systems.

CONCLUSIONS AND PHYSIOLOGICAL RELEVANCE

Evidence is accumulating to suggest that many types of ion channels are regulated, not only by growth factors through receptor PTKs but also by integrins through nonreceptor PTKs. Clearly, the study of interactions between integrins, or other adhesion proteins, and ion channels is in its infancy. Nevertheless, several studies already provide strong evidence that ECM proteins and other integrin ligands can modulate ion channels through nonreceptor PTKs. Given the increasing evidence for synergy between receptor PTKs and integrin signaling pathways, this suggests a paradigm whereby growth factors and cell–cell and cell–substrate interactions might acutely regulate cell function through ion channels.

The relevance of most of these interactions remains to be determined. It is possible that

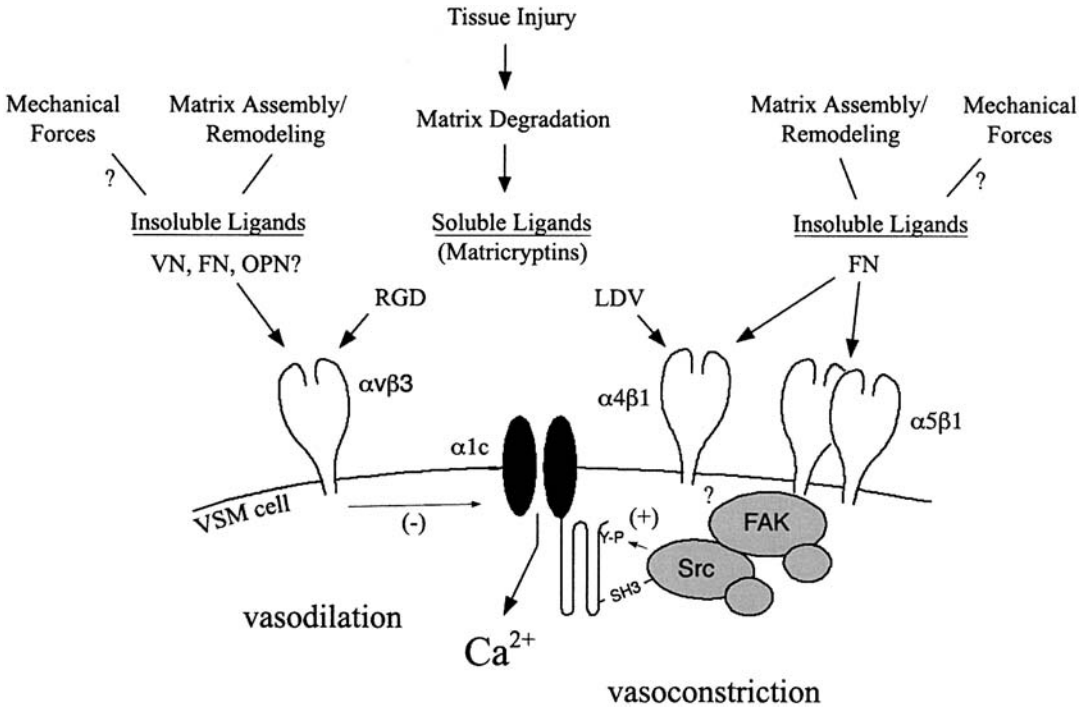

Fig. 1. Integrative scheme for regulation of L-type Ca^{2+} channel in VSM by ECM and integrins. *See* text for details. VN: vitronectin; FN: fibronectin; OPN: osteopontin; VSM: vascular smooth muscle; Src: c-Src; FAK: focal adhesion kinase; Y-P: tyrosine phosphorylation site; α1c: α1 pore-forming subunit of L-type Ca^{2+} channel.

integrins only regulate ion channels under pathological conditions such as tissue ischemia, reperfusion injury, wound repair, and vascular wall remodeling. However, the well-established role for integrins in transducing mechanical force across the cell membrane *(30,178,179),* leaves open the possibility that mechanical forces may be constantly transmitted through integrins or other cell-adhesion proteins to regulate ion channels. Both possibilities need to be more thoroughly investigated with electrophysiological studies.

Figure 1 shows an illustration of the possible interactions between ECM integrins and the L-type Ca^{2+} channel in vascular smooth muscle. These mechanisms summarize the studies discussed in the body of this review. Signaling pathways involving at least three different integrins have the potential to regulate this channel. Soluble integrin ligands (matricryptins) that might be formed from the degradation of ECM (e.g., collagen) during tissue injury responses (e.g., wounding, ischemia/reperfusion, neutrophil invasion) can act on αvβ3 and α4β1 integrins to inhibit or potentiate, respectively, L-type Ca^{2+} current *(97).* Inhibition would lead to vasodilation, because the channel is partially open at the resting potential of VSM; potentiation of current would lead to vasoconstriction. In addition, insoluble (bound) ligands, such as matrix proteins, may also constitutively regulate the channel; vitronectin (VN), through αvβ3, may exert inhibitory signals; FN, through α5β1, may exert excitatory signals. Both acute and chronic conditions might alter the balance of these signaling mechanisms. For example, mechanical forces (stretch, shear stress) might modulate interactions between FN and α5β1 or interactions between VN and αvβ3. Likewise, long-term

changes in the assembly or expression of ECM or integrins could shift the balance of these signaling mechanisms that converge on the primary Ca^{2+} influx pathway in VSM.

Given the above discussion, the potential exists for a number of common pathophysiological states to demonstrate involvement of an extracellular matrix–integrin–ion channel axis. Examples pertaining to vascular physiology include dysfunctions associated with diabetes mellitus and hypertension. Both states have been shown to be associated with alterations to the extracellular matrix component of the blood vessel wall, including increased deposition of fibronectin *(180–183).* An altered complement of matrix proteins could conceivably alter cell signaling initiated through integrin binding and/or by alterations in the mechanical properties of the vessel wall. In experimental diabetes, accumulation of matrix proteins in the arteriolar wall has been suggested to decrease distensibility and, consequently, impair smooth-muscle Ca^{2+} entry and mechanotransduction *(183).* Similarly, impaired shear-stress-mediated mechanotransduction in endothelial cells could limit Ca^{2+} entry via pathways such as store-depletion-dependent Ca^{2+} entry, thus contributing to the decreased nitric oxide production seen in hypertension *(184).*

In addition to an involvement of the extracellular matrix component, *per se,* disorders such as hypertension have been shown to be associated with altered expression of integrins. Arterial vessels from the spontaneously hypertensive rat exhibit age-dependent increases in $\alpha V\beta 3$ and $\alpha 5\beta_1$ integrins *(181,185).* As voltage-gated Ca^{2+} channels appear to be regulated by activation of such integrins (see the section Interactions Between Ion Channels and Integrins), it is an attractive hypothesis that altered vascular reactivity and, hence, resistance, in hypertension may result from altered integrin expression. Further studies should be directed at understanding the functional consequences of pathological alterations in interactions among extracellular matrix proteins, integrins, and ion channels.

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NOTE ADDED IN PROOF

HVA current in molluscan neurons is modulated by FN and RGD peptides, with low doses of cRGD reducing current and high doses increasing current *(186).*

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