Signaling Mechanisms Underlying the Vascular Myogenic Response

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Davis, Michael J., and Michael A. Hill. Signaling Mechanisms Underlying the Vascular Myogenic Response. *Physiol. Rev.* 79: 387–423, 1999.—The vascular myogenic response refers to the acute reaction of a blood vessel to a change in transmural pressure. This response is critically important for the development of resting vascular tone, upon which other control mechanisms exert vasodilator and vasoconstrictor influences. The purpose of this review is to summarize and synthesize information regarding the cellular mechanism(s) underlying the myogenic response in blood vessels, with particular emphasis on arterioles. When necessary, experiments performed on larger blood vessels, visceral smooth muscle, and even striated muscle are cited. Mechanical aspects of myogenic behavior are discussed first, followed by electromechanical coupling mechanisms. Next, mechanotransduction by membrane-bound enzymes and involvement of second messengers, including calcium, are discussed. After this, the roles of the extracellular matrix, integrins, and the smooth muscle cytoskeleton are reviewed, with emphasis on short-term signaling mechanisms. Finally, suggestions are offered for possible future studies.

I. INTRODUCTION

A. Definition

Blood vessels respond to transmural pressure elevation with constriction and to pressure reduction with dilation. This behavior, termed the myogenic response, is inherent to smooth muscle and independent of neural, metabolic, and hormonal influences. It is most pronounced in arterioles but can be demonstrated occasionally in arteries, venules, veins, and lymphatics (186).

When longitudinal comparisons are made among arterioles of a given vascular network, an inverse relationship between vessel size and myogenic responsiveness is consistently observed (55), although the cerebral circulation may be an exception to this rule (278).

Three examples of myogenic behavior are illustrated in Figure 1. *1*) Figure 1*A* shows the prototypical myogenic response of a cannulated arteriole to a step increase in pressure. After the pressure step, an initial, passive distension is followed by two phases of constriction; upon release of the pressure step, the arteriole transiently collapses, then dilates. *2*) In addition to this reaction, arte-
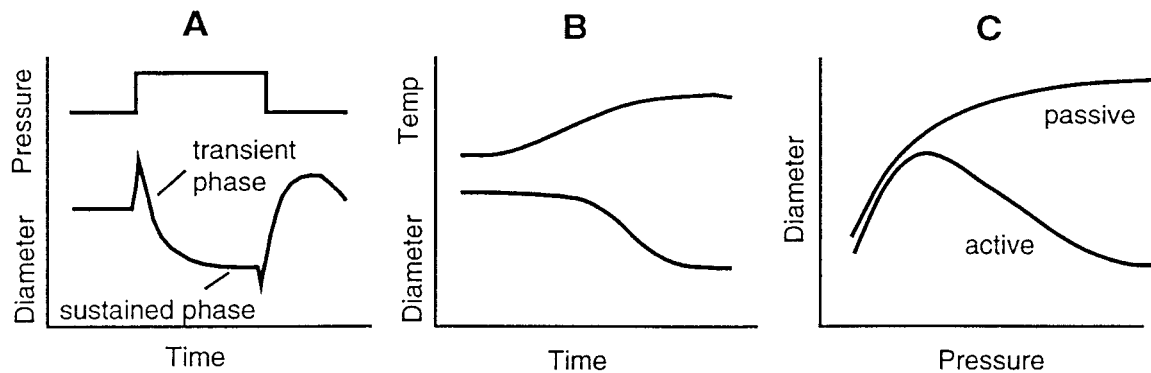
FIG. 1. Examples of myogenic behavior in arterioles (for explanation, see text). Approximate duration of pressure step in *A* is 2 min. Quantitative scales for time, pressure, and diameter depend on vessel type, size, species, and method of study. For representative examples, see References 64 (*A*), 211 (*B*), and 55 (*C*).

rioles typically develop and maintain some degree of active force at their normal intravascular pressure. This is depicted in Figure 1*B* by the diameter response of an isolated arteriole that is maximally dilated after being cannulated and pressurized, but then spontaneously constricts to ~50% of its passive diameter when temperature is raised from 22 to 37°C. The constriction would typically be maintained for several hours. *3*) A third way in which myogenic behavior is defined can be illustrated by a graph of diameter versus pressure for an arteriole with (active) or without Ca^{2+} (passive) in the bathing media (Fig. 1*C*). The range of pressures over which the active diameter curve has a less positive slope than the passive curve is termed the myogenic range. In arteries, this slope may be only slightly less positive than that of the passive curve (231), but in arterioles, it can be quite negative (55). Additional descriptions of myogenic behavior in isometric preparations are discussed in section IIIB.

B. Historical Perspective

Discovery of the myogenic response is credited to Bayliss in 1902, when he recorded large increases in the volume of the dog hindlimb following release of brief aortic occlusions (14). Bayliss considered this response too rapid to be mediated by accumulation of metabolites and thought it reflected the same mechanism by which isolated arteries constricted following sudden distension. It is not usually appreciated that Bayliss' experiments were predated by a number of other studies, for example, by Jones in 1852 (190), Ostroumoff in 1868 (283), and Gaskell in 1881 (104). It was Bayliss, however, who clearly formulated the idea that a significant component of vascular tone could be determined by intravascular pressure.

Bayliss' ideas were challenged by Anrep (8), who believed the hindlimb response could be explained by metabolic factors. Partly because of Anrep's persuasive

arguments, relatively little work on the myogenic response was performed over the subsequent 45 years. Notable exceptions to this include the work of Fog (89), Forbes et al. (93), Wachholder (353), Klemensiewicz (204), and Bürgi (36). Despite these studies, which tended to confirm Bayliss' original findings, the majority of workers in this field attributed local vascular regulation primarily to chemical and neural mechanisms until Folkow demonstrated that denervated preparations developed pressure-dependent vascular tone (90) and that autoregulation of blood flow was due in part to a nonneural, pressure-dependent mechanism (91).

Due in large measure to Folkow's work, Selkurt and Johnson (316) in the 1950s, and Johnson (183) in the 1960s, studied the myogenic response using increasingly sophisticated whole organ techniques and concluded this mechanism could account for significant vascular resistance changes in vivo. Concurrently, Burnstock and Prosser (37) demonstrated that strips of nonvascular smooth muscle reacted to quick stretch with active force generation, and Sparks (331) described the same phenomenon in vascular smooth muscle (VSM). In the late 1960s, Johnson (184) and Wiederhielm (370) pioneered the application of techniques for quantitating the myogenic response in the microcirculation, which led to intense investigation over the subsequent decade (11, 33, 188). Development of isolated vessel techniques in 1981 (75) enabled more careful quantitation of the myogenic response and its underlying mechanisms, first in small arteries (132, 279) then in arterioles (175, 211), where the effects of pressure could be clearly distinguished from flow, metabolic, neural, and endothelial influences.

C. Scope of This Review

This review attempts to summarize and synthesize what is known regarding the cellular mechanisms under-

lying the vascular myogenic response. Because myogenic behavior is only one aspect of VSM mechanotransduction, thorough treatment of that topic would include a discussion of mechanical effects on secretion and growth as well as contractile function, which is beyond the scope of this review (see Ref. 278 for more general coverage). The reader is referred to Johnson's comprehensive review on the myogenic response for information on studies before 1979 (186), to reviews of relevant microcirculatory studies from 1979 to 1990 (55, 61), and to several shorter reviews on myogenic mechanisms by workers in this field (23, 24, 51, 137, 241, 278). The role of the endothelium has been reviewed previously (24, 241). Because isolated vessel preparations have provided the most definitive information regarding cellular mechanisms involved in the response, the present article emphasizes in vitro studies using blood vessels and single VSM cells performed mostly after 1980. Particular attention is given to recent work using biochemical and electrophysiological techniques and to data collected from arterioles; however, relevant data from conduit arteries are cited when specific information about microvessel function is missing.

II. PHYSIOLOGICAL SIGNIFICANCE

In the vascular system, the myogenic response has been proposed to participate in a number of physiologically important functions. The two most important of these are *1*) establishment of basal vascular tone and *2*) autoregulation of blood flow and capillary hydrostatic pressure. Other roles for the myogenic response have been discussed in detail elsewhere (186, 187).

A. Basal Vascular Tone

Basal vascular tone is a prerequisite for dilator influences. It establishes an underlying arteriolar constriction, a "regional blood flow reserve" (92), upon which other control mechanisms produce vasodilation or vasoconstriction.

Both Bayliss and Folkow suggested that basal tone might result from myogenic mechanisms. This conclusion derived, in part, from the pressure-dependent resistance to flow observed in denervated whole organ preparations (91, 245, 361). A common finding in microcirculatory studies is that responsive and stable preparations are associated with the development of spontaneous tone in nearly all arterial vessels less than 150 μm ID; the tone is easily compromised by excessive levels of anesthesia, extensive surgical manipulation, or trauma (60, 75). In isolated artery and arteriole preparations, the level of tone is often comparable to that observed in the same vessels in vivo and rarely develops if the vessels are not pressurized to a physiological level (55, 60).

In addition to the effect of a static pressure head, another component of vascular tone may be related to pulsatile pressure. A classic study of isometric portal vein by Johansson and Mellander (180) demonstrated both static- and rate-sensitive components in the response to stretch, evident in the electrical and mechanical activity of the preparation. A similar effect was observed in the cerebral artery, although maximum sensitivity occurred at a much different rate of stretch than portal vein (259). In studies of isolated pump-perfused organs, switching from static to a pulsatile pressure produced an increase in calculated vascular resistance of the perfused organs (303, 319). Mellander (244) suggested these responses reflected a rate-sensitive myogenic component that is essential for the development of normal vascular tone. However, experimental support for this idea is weak because isolated arteries and arterioles usually develop tone comparable to that observed in vivo when connected to a static pressure head (55). Moreover, switching from static to pulsatile pressure produces no significant change in the diameter of cannulated arterioles (57) or small arteries (113). Thus the response of isolated organs to pulsatile perfusion may involve more than simply a pressure effect, possibly due to release of endothelium-derived vasoactive factors (91, 167).

B. Autoregulation of Flow and Pressure

The myogenic response has also been postulated to play a central role in the maintenance of constant blood flow and capillary hydrostatic pressure (P_c) during variations in systemic arterial pressure. Whole organ data collected by Johnson (185) suggested that changes in arterial inflow or venous outflow pressure produced changes in arterial resistance that would serve to minimize changes in capillary hydrostatic pressure. Mellander and colleagues (26, 177) demonstrated that tissue volume of cat hindlimb skeletal muscle was nearly constant over a wide range of systemic arterial pressures (30–170 mmHg). Under the assumption that a constant tissue volume reflected a constant P_c, it was concluded that "autoregulation of P_c" was achieved through myogenic adjustments of arteriolar tone. However, this conclusion assumed that other Starling forces were not involved in control of tissue volume and that other local regulatory mechanisms did not contribute significantly to the vascular resistance adjustments (31, 52, 112). Even though whole organ techniques are subject to significant limitations (61), direct measurements of P_c in microcirculatory preparations have been unable to completely resolve this issue (see Ref. 61 for review).

It is important to note that the contribution of myogenic mechanisms to P_c regulation might depend on whether a selective change in arterial or venous pressure

occurs or whether both pressures change equally (61, 115). In the case of perfusion pressure reduction, microcirculatory data suggest that partial P_c regulation does occur in some tissues but that a significant fraction of that regulation may be contributed by factors other than the myogenic response (31, 53, 111, 327). However, when arterial and venous pressures are equally raised or lowered, as during postural changes, the contribution of the myogenic response to P_c regulation appears to be much greater (52, 92, 224); this may be related to the position at which an arteriole normally rests on its pressure-diameter curve (64) or to the fact that endothelial-derived nitric oxide (the release of which is altered if flow changes along with pressure) is a potent antagonist of myogenic tone (210).

III. CONCEPTUAL BASIS FOR MYOGENIC BEHAVIOR

A. Length-Dependent Activation

Vertebrate muscle, including VSM, exhibits length-dependent regulation of force, such that peak force development due to contractile protein interaction is generated at an intermediate, optimal sarcomere length. A classic concept in cardiac and smooth muscle mechanics is the distinction between active force development due to initial length (preload) and that due to activation (inotropy, contractility) (179). However, over the past 20 years, experiments on cardiac muscle have clearly demonstrated that muscle length itself influences contractility (217), leading to the conclusion that preload and ionotropic state are not independent regulators of active force (5, 179). There is now general agreement that the relative steepness of the ascending limb of the length-active tension relationship in cardiac muscle reflects a progressive shift to increasing levels of activation with increasing length (97). That relationship broadens, as predicted, at high (fixed) levels of Ca^{2+} in skinned preparations of cardiac muscle (84), in contrast to skeletal muscle where the length-active tension relations of intact and skinned (at saturating Ca^{2+} concentration) preparations are superimposable (5).

In blood vessels, initial length is a well-known modulator of agonist sensitivity (110, 122, 133, 138, 231, 271, 293, 332, 341, 347). Conversely, agonists often potentiate myogenic responsiveness (83, 242, 299, 334, 335, 347). Thus it is likely that agonist- and stretch-activated signaling pathways overlap. Agonists such as norepinephrine (NE) are positive inotropic agents for smooth muscle. Likewise, a myogenic constriction is considered to represent an enhanced smooth muscle activation state (175, 186). Despite the plausibility of this idea, experimental support for it is mostly indirect. Johnson (184), and others

(59), analyzed the behavior of in vivo arterioles following step changes in perfusion pressure and concluded that smooth muscle must shift to a higher active length-tension curve in response to elevated pressure. However, those studies were limited in that active and passive components of wall tension could not be distinguished. Nevertheless, a shift in activation state is supported by isolated arteriole experiments showing that maximal velocity of arteriolar muscle shortening increases with pressure over the myogenic range of the vessel (54).

B. Isometric Versus Isobaric Preparations

The two experimental approaches typically used to quantitate the vascular myogenic response, isometric and isobaric protocols, have often led investigators to different conclusions with regard to mechanisms (74). Perhaps part of the reason for this is that the magnitude, time course, and direction of vascular wall tension changes in isometric contractions of vascular rings and strips are very different from those in isobaric contractions of cannulated arterioles and arteries. These differences are illustrated in Figure 2. In isometric preparations, stretch activation is represented by a slower, secondary increase in tension after stretch. By this definition, skeletal (301), cardiac (5), and smooth muscle (37) all exhibit stretch
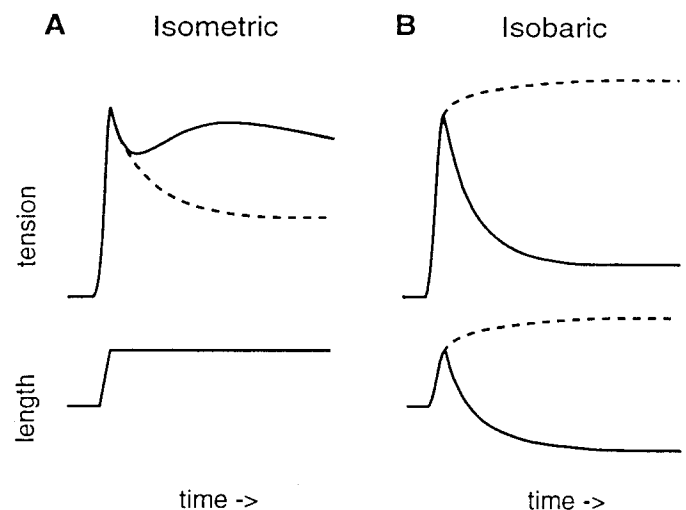

FIG. 2. Comparison of protocols for determining stretch activation of a blood vessel. *A*: in an isometric experiment, myogenic activity is identified by a secondary increase in tension observed in response to a rapid length increase. Dotted line shows estimated stress-relaxation curve in Ca^{2+}-free solution; difference between tension trace and this curve (or, more conservatively, a horizontal line drawn from beginning of secondary response) is defined as myogenic tone (259). *B*: in an isobaric experiment, a rapid pressure increase elicits a sharp rise in tension and length, but as arteriole constricts (in this case below control diameter), tension is reduced. Myogenic tone is defined as difference between maximum (dotted line refers to Ca^{2+}-free) and minimum diameter (194) [or, more conservatively, between control and minimum diameter (128, 231)].

activation. In isobaric preparations, activation of the contractile apparatus following a pressure increase results in a constriction that secondarily reduces total wall tension. However, this reduction is achieved by a decrease in passive tension, which more than compensates for the increase in active tension due to activation of the contractile machinery. Because cannulated vessels often respond with sustained constrictions to pressure elevation, it has been suggested that wall tension, rather than smooth muscle cell length, may be regulated during a myogenic (isobaric) constriction (186, 208). If contractile and sensor elements were arranged in series, a tension-control system would require only modest gain to perfectly regulate diameter, and sustained constrictions could be achieved in the face of elevated transmural pressure (as shown in Fig. 2*B*). Although widely accepted, the wall tension hypothesis has been difficult to test experimentally, and support for it derives chiefly from correlative evidence (38) and logical arguments (347).

There are a number of other differences between the behavior of isometric and isobaric preparations. Isometric preparations typically show maximal stretch activation in response to large, and perhaps unphysiological, changes in length. For example, secondary force production is maximal for length increases to 149% of control in rat mesenteric artery (347), 150% of control in rabbit basilar artery (259), and 140% of control in pig coronary artery (290). In contrast, isobaric preparations show maximal constrictions in response to much smaller length changes (<25% of control) (55, 347), even in the absence of detectable distension (64). In vessels of the same size and type, isobaric preparations exhibit different agonist sensitivity than isometric preparations (77, 174, 225, 313, 347), as well as differences in the magnitude of agonist-induced VSM depolarization (313). Interestingly, most of the evidence for stretch-activated Ca^{2+} entry through a non-voltage-dependent pathway comes from isometric preparations (23, 168, 169, 215, 216, 379) (see sect. IV*A6*).

One phenomenon confirmed by both isometric and isobaric preparations is shortening deactivation. In isometric protocols, shortening deactivation is the disproportionate decline in force relative to length observed in actively contracting muscle (5). In isotonic release protocols, it is a depression in shortening velocity in response to a step decrease in length (240). Shortening deactivation is observed in both large and small vessels with (175) or without myogenic tone (29, 126, 300) as well as in nonvascular smooth muscle (121), cardiac muscle (5), and skeletal muscle (343). Jackson and Duling (175) demonstrated this phenomenon in pressurized arterioles. The mechanism of shortening deactivation has not been elucidated but is thought, in other muscle types, to represent changes in mechanisms controlling intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) as well as changes in myofilament Ca^{2+} sensitivity (84). In arterioles, shortening deactiva-

tion is more pronounced with intrinsic tone than with agonist-induced tone (175). It seems reasonable to conclude that the myogenic constrictions and dilations characteristic of pressurized vessels reflect the same underlying mechanisms represented in isometric rings by stretch activation and shortening deactivation, respectively.

In summary, although some authors have made distinctions between the terms *myogenic response*, *stretch activation*, *pressure-dependent contraction*, *myogenic tone*, *basal tone*, *spontaneous tone*, and *intrinsic tone* (24, 278, 280, 335, 350), the limited amount of quantitative information available in any one tissue restricts the usefulness of such an approach at the present time. For the purposes of this review, we assume that all of the terms above describe cellular processes with similar underlying mechanisms. Doubtless, some of the discrepancies in the literature regarding mechanisms will be resolved when this issue is addressed systematically.

IV. TRANSDUCTION MECHANISMS

Bohr and colleagues (345) have been credited (259, 278) with the initial suggestion that the myogenic response might reflect an improved excitation-contraction coupling resulting from membrane depolarization and increased Ca^{2+} permeability. This idea was based on simultaneous measurements of tension and membrane potential in taenia coli by Bülbring (35), coupled with the demonstration of Ca^{2+}-dependent myogenic tone in resistance vessels (345). Currently, the prevailing thought is that a myogenic contraction is initiated by VSM depolarization (mechanisms not yet agreed upon) which then regulates Ca^{2+} entry through voltage-gated Ca^{2+} (VGC) channels (241). This basic mechanism, as proposed in Figure 3, is almost certainly modulated by a number of
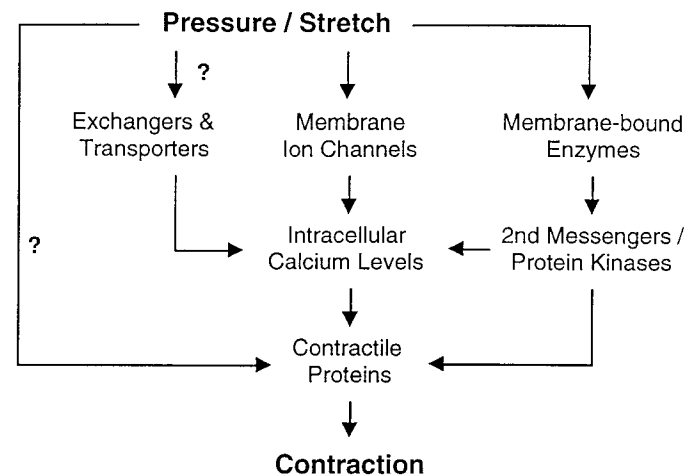
FIG. 3. Putative membrane mechanisms involved in vascular smooth muscle mechanotransduction (for details, see text).

intracellular signaling mechanisms. The experimental evidence for each of these components is discussed in section IVA. These discussions will almost exclusively focus on mechanisms activated in response to pressure elevation (or increased stretch), but it is assumed that the same mechanisms are modulated in the opposite way in response to pressure reduction.

A. Electromechanical Coupling

1. Depolarization of VSM

Since Bülbring's original study (35), much additional evidence is now available to suggest that membrane depolarization plays a central role in the response of smooth muscle to stretch (46, 135, 205, 250, 333, 347, 368). Resting potentials of VSM cells typically range from -60 to -75 mV in unpressurized small arteries and arterioles (150, 267), and graded depolarization is observed as pressure is increased [although membrane potential usually cannot be measured continuously as pressure is changed due to movement of the vessel wall (368)]. At physiological pressures, resting potentials range from -40 to -60 mV (78, 132, 266, 267, 394). The work of Harder and others has demonstrated that pressure- or stretch-induced depolarizations occur in a number of different vascular (132, 205, 328, 368) and nonvascular (46, 212, 363) smooth muscle preparations. Figure 4A summarizes data from cat cerebral artery myocytes as pressure was changed from 0 to 160 mmHg, showing that a graded, 20-mV depolarization occurred at pressures between 30 and 110 mmHg, which was the range associated with myogenic tone. Pressurization also increased the rate of action potential firing (132, 328). Both depolarization and constriction were attenuated when the extracellular Ca^{2+} concentration was reduced but were unaffected by tetrodotoxin (to block voltage-gated Na^+ channels) or phentolamine (to block the action of NE released from nerve terminals) (132).

Single smooth muscle cells also exhibit graded depolarization when longitudinal stretch is applied (Fig. 4B). This observation was first recorded in pig coronary VSM (58), then in bladder myocytes (363), and more recently in mesenteric artery myocytes (317). When coronary artery myocytes were stretched 25% beyond their slack length, a 35-mV peak depolarization (from a resting potential of -52 mV) was recorded (58, 381). This degree of stretch was equivalent to that seen in isolated arterioles rapidly pressurized from the minimum to the maximum of their myogenic range (64). In single-cell preparations, stretch was also associated with initiation of action potentials or an increase in action potential firing rate (363).

Despite the above evidence, it has been difficult to establish a definitive cause-and-effect relationship between membrane depolarization and myogenic responsiveness. In preparations without inherent myogenic tone, KCl application is often used to mimic myogenic depolarization, yet the behavior of KCl-activated and spontaneously myogenic preparations is often different, leading to the conclusion that simple, electromechanical coupling cannot fully account for myogenic behavior and that other mechanisms, e.g., changes in Ca^{2+} sensitivity, must be involved (348, 367). Two types of experiments have been used to minimize pressure-induced depolarization. *1*) Vessels permeabilized with saponin or α-toxin, in which no membrane potential can be generated, fail to demonstrate myogenic tone or constrict to pressure elevation (80, 178, 239, 400). *2*) Depolarization of normal arterioles with KCl should theoretically prevent stretch-induced membrane potential changes when sufficiently high concentrations of extracellular K^+ ($[K^+]_o$) are reached, because the K^+ equilibrium potential approaches 0 mV. Yet, intermediate increases in $[K^+]_o$ could shift the VSM membrane potential to a more optimal point on the open probability versus membrane potential relationship for VGC channels, thereby enhancing Ca^{2+} entry through that pathway (this effect has been demonstrated in pial arteries; Ref. 108). When the sustained phases of myogenic contractions are analyzed, KCl consistently reduces myogenic responsiveness, as indicated by the increased values of calculated myogenic index in Table 1. However, when individual records are shown, it is clear that the initial constrictor phase of the response is retained (239, 369). This differential action of KCl probably reflects differences in the underlying mechanisms involved in the two components
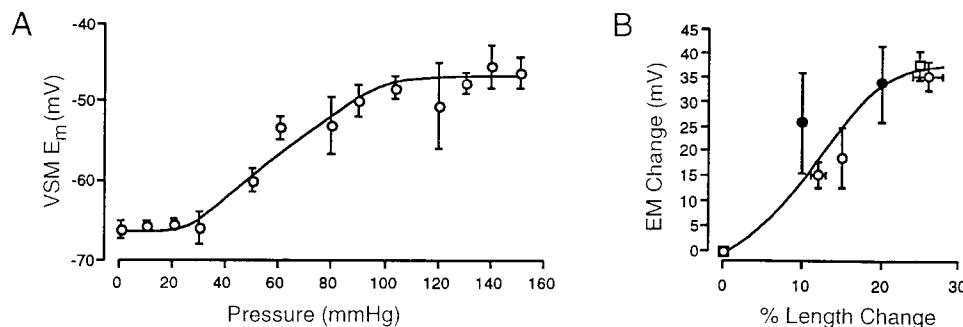
FIG. 4. Pressure- and stretch-induced depolarization of smooth muscle in pressurized arteries (A) and longitudinally stretched single cells (B). VSM, vascular smooth muscle; E_m, membrane potential. [Data in A are redrawn from Harder (132) by computing averages of individual data points. Data in B are redrawn from Davis et al. (58) (open circles), Wellner and Isenberg (363) (solid circles), and Setoguchi et al. (317) (open square with length estimated).]

TABLE 1. *Comparison of KCl effects on myogenic responses of arteries and arterioles*

$[K^+]_o$, mM	$[Ca^{2+}]_o$, mM	Isosmotic Substitution?	Tissue	Maximum Diameter, μm	Amount of Spontaneous Tone, %	Initial MI	MI After KCl	Reference No.
35	2.0	Yes, for Na$^+$	Hamster cheek pouch	81	34	-0.12	0.18	175
70	2.0	Yes, for Na$^+$	Hamster cheek pouch	81	34	-0.12	1.19	175
40	?	Yes, for Na$^+$	Rat cremaster, in vivo	126	~0	0.27[a]	0.20[a]	242
80		Yes, for Na$^+$	Rat cremaster, in vivo	126	~0	0.27	0.51[a]	242
30	2.0	Yes, for Na$^+$	Rabbit basilar, isometric	~450		100%[b]	590%[b]	259
100	100	Yes, for Na$^+$	Rabbit basilar, isometric	~450		100%[b]	90%[b]	259
132	2.5	Yes, for Na$^+$	Pig coronary artery, isometric	?	0	?[c]	$+$[c]	290
125	0.06	Yes, for Na$^+$	Rat cerebral artery	180	~28	-0.02	0.35[d]	239
32	1.6	?	Rat mesenteric artery	?	~0	-0.29[f]	0.29[f]	347
36	1.6	Yes, for Na$^+$	Rat mesenteric artery	329	~0	0.09[e]	0.48[e]	369

All preparations were cannulated vessel segments unless otherwise stated. Myogenic Index (MI) was calculated from MI = $100(\Delta d/d_{init})/\Delta P$, where d is diameter and P is pressure, when possible, as defined in Ref. 279. Negative values indicate constriction in response to increasing pressure (values computed at pressures near estimated physiological pressure). Amount of spontaneous tone is tone at estimated physiological pressure in PSS. $[K^+]_o$, extracellular K$^+$ concentration; $[Ca^{2+}]_o$, extracellular Ca^{2+} concentration. [a] Estimation based on assumption that 50% of change in box pressure was transmitted to arteriole. [b] MI could not be calculated; responses are percent of control. [c] MI could not be calculated; response in Tyrode solution was not given as a reference, but stretch activation persisted in KCl solution. [d] Change in MI does not reflect fact that initial transient was not changed in PSS (34 μm) vs. KCl (36 μm). [e] MI was estimated from normalized values of cross-sectional area. [f] MI was estimated from normalized values of cross-sectional area in the presence of 0.3 μM norepinephrine.

of the response. In addition, KCl substitution protocols may have unanticipated actions on ion transporters or contractile protein sensitivity to Ca^{2+} (276, 388).

2. Mechanosensitive channels

Because the resting potential of smooth muscle is determined to a large extent by K$^+$ (267), stretch-induced depolarization could be explained by activation of mechanosensitive (MS) ion channels promoting Na$^+$ or Ca^{2+} influx, Cl$^-$ efflux, or inhibiting K$^+$ efflux (Fig. 5). Sodium-permeable MS channels were first described in cultured skeletal muscle cells (119) and have since been found in a number of cell types, including smooth muscle (Table 1). Likewise, MS K$^+$ and Cl$^-$ channels have also been described in several cell types (254). On the basis of both theoretical considerations and experimental evidence, it

is thought that MS channel gating is controlled by forces transmitted through the cytoskeleton (see sect. IV*E* and Ref. 308). Mechanosensitive channels appear to be involved in many aspects of cell function, but it is not clear whether different mechanical stimuli activate different classes of MS channels. Stretch-activated currents, such as those recorded in muscle cells (119, 201), are often contributed by nonselective cation channels with characteristics similar to currents in mechanotransduction organs (274), whereas volume-activated currents are typically carried by Cl$^-$ (125, 307). Some studies have distinguished between volume- and stretch-activated currents in the same cell type (163, 310, 351, 396).

Patch-clamp techniques have been used to identify and characterize a number of MS channels in muscle cells. Because cells in intact vessels are electrically cou-
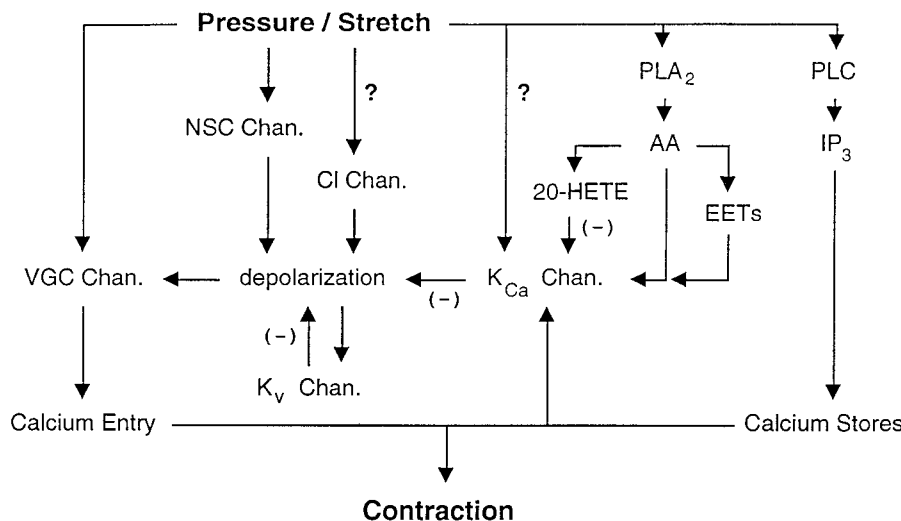
FIG. 5. Putative ion channels involved in vascular smooth muscle mechanotransduction (for details, see text). PLA$_2$, phospholipase A$_2$; PLC, phospholipase C; IP$_3$, inositol 1,4,5-trisphosphate; AA, arachidonic acid; 20-HETE, 20-hydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acid; VGC, voltage-gated Ca^{2+} channel; K$_{Ca}$, Ca^{2+}-activated K$^+$ channel; K$_v$, voltage-gated Ca^{2+} channel; NSC, nonselective cation.

pled and surrounded by matrix proteins, single cells must be harvested for patch-clamp studies by enzymatic digestion of arteries and arterioles. Mechanosensitive channels relevant to vascular and visceral smooth muscle are summarized in Table 2. In each case, the channels were recorded from single cells using one of the three single-channel recording modes and were activated by suction applied to the rear of the patch pipette. It is apparent from this list that MS channels with a wide range of permeabilities have been identified in smooth muscle, although the most commonly reported type is a nonselective cation channel (NSC).

Are MS currents artifacts? In 1991, Morris and Horn (255) published a controversial paper in which a number of substantial mechanical stimuli failed to elicit whole cell MS currents from *Aplysia* neurons; because that cell type had been shown to contain a high density of stretch-activated K^+ channels (322), it was predicted that activation of even a small fraction of the MS channel population would produce easily detectable, whole cell current. These negative results led to the conclusion that MS current might be an artifact of single-channel recording. However, most investigators in this field continue to accept MS current measurements as valid, for a number of reasons summarized previously (129, 241, 337, 338). A compelling argument against the artifact hypothesis derives from the observation that multiple studies in different muscle preparations have now demonstrated reversible, graded, whole cell, MS currents. In addition, MS channels from *Escherichia coli* have been cloned (337). Nevertheless, the physiological roles of these channels, particularly as they relate to force transduction in muscle, remain to be established.

3. Nonselective cation channels

The first recordings of a MS channel in smooth muscle were made by Kirber et al. (201) in myocytes isolated from toad stomach (Table 2). In cell-attached and inside-out patch recording modes, increases in membrane stretch activated a cation channel permeable to K^+, Na^+, and Ca^{2+}. The channel exhibited a sigmoidal increase in open probability with increasing pipette suction (364). Channels with similar characteristics were recorded in myocytes isolated from coronary artery (58), mesenteric arterioles (56, 275), and urinary bladder (362). Single-channel conductances ranged from 30 to 40 pS (58, 201, 362) for monovalent cations. In the presence of Ca^{2+}, the channels exhibited slight inward rectification (201, 362) and reduced monovalent cation conductance (201), suggesting a Ca^{2+}-dependent inactivation mechanism. The channels were blocked by Gd^{3+} (275, 362). Although the opening of a cation channel by membrane stretch would conceivably depolarize a cell and recruit voltage-gated Ca^{2+} channels (58, 201), its physiological role cannot be determined from single-channel measurements alone.

To better address the issue of physiological relevance, a method was developed for recording whole cell currents during VSM stretch (58). With the use of two to three modified patch pipettes, single myocytes could be stretched in the longitudinal direction up to 30% above the slack length of the cell. This stimulus consistently elicited an inward current, whereas, in current-clamp mode, single-cell stretch produced depolarization. Subsequently, stretch-activated, whole cell currents (Table 3) and/or depolarizations (Fig. 3B) were confirmed by Wellner and Isenberg (363, 364) and Setoguchi and co-workers (275, 317) in smooth muscle as well as in other types of muscle (163, 310) and nonmuscle cells (123, 397). In all three smooth muscle studies, the reversal potential for whole cell current (after excluding the contribution of secondary K^+ current) was between 0 and -20 mV (58, 275, 363), varied with intracellular Na^+ concentration ($[Na^+]_o$) (317), and was not altered by changes in extracellular Cl^- concentration (58, 317); these characteristics are consis-

TABLE 2. *Single-channel mechanosensitive currents in smooth muscle*

Selectivity	Conductance, pS	Modulators	Preparation	Reference No.
Cations	~30 for Na^+ with Ca^{2+}		Toad stomach	201
Cations	~40 for Na^+ with no Ca^{2+}		Rabbit pulmonary artery	198
K^+	260 for K^+	Calcium	Rabbit mesenteric artery	71
K^+	20 for K^+	Fatty acids	Toad stomach	277
Cations	~64 for Na^+ with no Ca^{2+}	Voltage and AlF_3	Toad stomach	152, 153
K^+	20 for K^+	Flow and $[Ca^{2+}]_o$	Toad stomach	200
Nonselective	62 for Na^+, K^+, Cl^-		Cultured mesangial cells	49
Divalent cation	21 to Ba^{2+}		Cultured mesangial cells	44
Cations	~8 for Na^+	Stretch inactivated	Toad stomach	154
Cations	~40 for Na^+ with no Ca^{2+}		Guinea pig bladder	362, 364
Cations	~40 for Na^+ with no Ca^{2+}		Hamster mesenteric artery	56
K^+	270 for K^+	Calcium	Rabbit pulmonary artery	199
K^+	201 for K^+	Cytoskeleton	DDT_1MF-2 cell line	79
Cations	32 for Na^+		Rat mesenteric artery	275
Cations	~40 for Na^+ with no Ca^{2+}		Pig coronary artery	58

Table 2 does not include channels activated by anisosmotic solutions.

tent with activation of a nonselective cation channel rather than a Cl^- or Ca^{2+} conductance. A component of the whole cell current was carried by Ca^{2+} (58, 317), but whole cell, MS cation currents could still be recorded in the presence of nicardipine to block VGC channels (317). Gadolinium blocked stretch-activated, whole cell current (275, 317, 363) and blocked stretch-induced depolarization (317). In two preparations, both single-channel and whole cell currents were shown to be Gd^{3+} sensitive (275, 363, 364). Whole cell MS cation currents were inhibited by increases in extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) and enhanced by decreases in $[Ca^{2+}]_o$, consistent with the modulatory effects of intracellular Ca^{2+} (consequent to Ca^{2+} influx) known to occur with other Ca^{2+}-permeable channels (201, 362, 390). Calcium entry through the MS cation channel produced a significant and sustained rise in $[Ca^{2+}]_i$ and contraction (64). Calcium influx caused inactivation of VGC channels and activation of tetraethylammonium (TEA)-sensitive K^+ channels (363). The interaction of this cation channel with other channels and signaling pathways in smooth muscle remains to be completely elucidated, but evidence suggests that its mechanosensitivity can be modulated by cAMP-dependent protein kinase (364). It is likely that this channel will be found to be regulated by other kinase systems as well [e.g., protein kinase C (PKC) regulates agonist-activated cation channels in gastric smooth muscle (197)].

Is activation of a MS cation channel necessary for initiation of the vascular myogenic response? This question has been difficult to answer because selective blockers are not available (for review, see Ref. 130). Dihydropyridines abolish myogenic tone, but do so by acting on VGC channels that are presumably downstream from cation channels in the signaling pathway (see sect. IV*A6*). Gadolinium, often thought to be a specific MS cation channel blocker (127), inhibits stretch-induced depolarizations and MS cation currents in isolated mesenteric artery myocytes (317) and eliminates myogenic tone in arterioles (401). However, Gd^{3+} also blocks VGC channels in some VSM cells at severalfold lower concentrations than required to block MS channels (25, 330), even though it may be more selective for MS channels over VGC channels in heart (131, 213). Aminoglycoside antibi-

otics such as streptomycin and neomycin have also been used to inhibit MS channels in other tissues (103, 378); these compounds block myogenic tone in rat cerebral arteries but only at doses higher than those required to block VGC channels (219, 246). Other purported MS channel blockers, such as *Grammostola spatulata* venom (270) and amiloride derivatives (130), have not been thoroughly tested on VSM channels, although amiloride and one of its analogs (at high doses) have been shown to inhibit myogenic tone (142). At this time, however, the lack of selective pharmacological tools to block MS cation channels has prevented determination of their potential role in the myogenic response.

As mentioned above, MS cation channels have been proposed to initiate contraction by depolarizing VSM cells past the threshold for activation of VGC channels (58, 201) and allowing Ca^{2+} entry through VGC channels to activate contractile proteins (241). Consistent with this idea is the observation that focal activation of MS cation channels (using pipette suction applied to a small membrane patch of a smooth muscle cell) elicits depolarization of the entire cell along with increases in $[Ca^{2+}]_i$ (118). Several other lines of evidence also support this hypothesis: *1*) stretch elevates $[Ca^{2+}]_i$ in single vascular muscle cells (62); *2*) pressure elevates VSM cell $[Ca^{2+}]_i$ in isolated arterioles (243); and *3*) VGC channel antagonists produce only a partial block of stretch-induced $[Ca^{2+}]_i$ increases in VSM (62, 402), whereas Gd^{3+} produces a complete block (402).

Sodium substitution experiments have been used to test the role of stretch-activated Na^+ entry mechanisms, but data from these experiments have produced confusing results. For example, in rat cerebral arteries, complete substitution of $Tris^+$ for Na^+ has no effect on the myogenic response (266), whereas in rabbit cerebral arteries, substitution of sucrose or *N*-methyl-D-glucamine for Na^+ inhibits the response (142). Sodium ionophores increase myogenic tone (142), but increasing $[Na^+]_i$ with oubain does not (257). In rabbit facial vein, decreases in $[Na^+]_o$ potentiate (rather than attenuate) myogenic tone (142), possibly by changing the sensitivity of the contractile system to Ca^{2+} (141). Although the latter studies are consistent with a regulatory effect of $[Na^+]_o$ on an extra-

TABLE 3. *Whole cell mechanosensitive currents in smooth muscle*

Selectivity	Block	Gating Stimulus	Preparation	Reference No.
Cations		Longitudinal stretch	Pig coronary artery	58, 381
Cations	Gd^{3+}	Longitudinal stretch	Guinea pig bladder	363, 364
Ca^{2+}	DHP	Inflation, swelling (+ voltage)	Rat basilar artery	218
Ca^{2+}	DHP	Inflation, swelling (+ voltage)	Rat posterior cerebral artery	239
Cations	Gd^{3+}	Inflation	Rat mesenteric artery	275
Cations	Gd^{3+}	Inflation, swelling	Rat mesenteric artery	317

Table 3 does not include currents activated by anisosmotic solutions alone. Block column does not necessarily mean block was specific for respective channel. DHP, the dihydropyridine (−)-202-791.

cellular stretch sensor, as proposed by their authors (141), interpretation of all these studies is complicated by the likelihood that Na^+ substitution has a profound effect on VSM ion transporters, such as the Na^+/Ca^{2+} exchanger and the Na^+-K^+ pump (see sect. IV*B*) (273, 296).

4. K^+ channels

Stretch-induced depolarization could result from inhibition of any of the various K^+ currents identified in smooth muscle (212), provided the channel was active when the vessel had basal vascular tone. A direct role for a K^+ channel in initiating the myogenic response has not been shown (see Table 2), but there is evidence that K^+ currents can and do counteract myogenic tone. Of the five major types of K^+ currents identified in VSM (267), three appear to play no significant role in the myogenic response: the inward rectifier K^+ channel, the ATP-sensitive (K_{ATP}) K^+ channel (294), and a novel K^+ channel (K_N) with kinetics similar to M-type neuronal current (81). Indirect evidence at first suggested a role for K_{ATP} channels in the response of the coronary microcirculation to a fall in perfusion pressure (209), but recently a more direct study failed to show a significant effect of K_{ATP} channel antagonists on isolated small arteries at any pressure (205). However, there is evidence that the other two types of channels, voltage-dependent K^+ (K_v) channels and Ca^{2+}-activated K^+ (K_{Ca}) channels, can provide potentially powerful repolarizing mechanisms to counteract stimuli resulting from VSM stretch. The K_v channels exhibit exponential increases in open probability upon depolarization and likely serve an important role in the repolarization of excitable cells (267). K_v channel blockers depolarize VSM cells in pressurized arterioles and augment myogenic tone (205). K_{Ca} channels are activated both by increases in $[Ca^{2+}]_i$ and by depolarization. The function of large-conductance K_{Ca} (BK) channels is particularly important to determine because activation of only a few channels would be sufficient to effect large changes in the membrane potential of a VSM cell (due to the high input resistance). For this reason, there is a substantial amount of information concerning the role of BK channels in the myogenic response.

It has been argued that stretch-induced depolarization could not be maintained unless an endogenous inhibitor of K_{Ca} channels is produced (137). This is because BK channels are activated by Ca^{2+} influx and by Ca^{2+} sparks [bursts of Ca^{2+} release from sarcoplasmic reticulum (SR)], producing pulses of outward current that substantially hyperpolarize the cell (265). Because myogenic tone is associated with both Ca^{2+} influx (9, 132, 147, 170, 215, 369) and Ca^{2+} release (62, 259), K_{Ca} current should be tonically activated when a blood vessel is at its normal pressure. Interplay between Ca^{2+}-permeable MS channels and K_{Ca} channels

has been demonstrated in other cell types (162, 321), and several lines of evidence support a similar interaction in VSM. For example, a K^+ conductance in rat saphenous arteries is activated by pressurization, enhanced by Ca^{2+} ionophores, and blocked by TEA (an antagonist with moderate specificity for K_{Ca} channels) (20); in dog basilar artery, there is a tight coupling between stretch-induced increases in Ca^{2+} influx and ^{86}Rb efflux (9); in longitudinally stretched smooth muscle cells, a TEA-sensitive voltage-gated K^+ current is activated secondary to activation of a MS cation current (363).

Further support for an important role of K_{Ca} channels comes from the observation that myogenic tone in cerebral arteries is enhanced by BK channel inhibition: at physiological levels of pressure, charybdotoxin (CTX), a specific inhibitor of BK channels, causes VSM depolarization and contraction, whereas at low pressures, it has little effect (34, 368). A depolarizing effect of CTX was also observed in pial arteries studied isometrically (108). In cerebral and renal arteries, Harder, Roman, and colleagues (136, 233, 398) have identified 20-hydroxyeicosatrienoic acid (20-HETE), a metabolite of arachidonic acid (AA) produced through cytochrome *P*-450 β-hydroxylation, as an endogenous and possibly tonic inhibitor of BK channels. Stretch-induced production of 20-HETE [possibly through phospholipase (PL) C or PLA_2 pathways] could thereby sustain or even initiate myogenic responses. Of course, epoxyeicosatrienoic acids are also produced from oxidative metabolism of AA by cytochrome *P*-450 epoxygenase, and several of these products have been shown to activate, rather than inhibit, BK channels (165). In support of a role for 20-HETE, Wesselman et al. (368) found that BK channels were necessary for the pressure-induced response of rat mesenteric arteries treated with NE (368): inhibition of BK channels with CTX caused a flattening of the pressure-diameter relationship. The authors concluded that pressure may induce depolarization and myogenic contraction by closure of BK channels. The use of (reputedly) selective inhibitors has also confirmed a role for 20-HETE in some (368) but not all (40, 324) preparations. However, a ubiquitous role for 20-HETE and other related molecules depends (in part) on the demonstration that BK channels are tonically active in arteries and arterioles with normal tone: this is observed in some (34, 108, 265, 268), but not all, blood vessels (176, 229, 287, 393), although these differences may be due to the membrane potential-dependent characteristics of CTX block (96) rather than differences in the relative importance or expression of the BK channel.

5. Cl^- channels

Under the proper conditions, Cl^- channel activation is another potential mechanism to explain stretch-in-

duced depolarization of VSM. Chloride channels have been implicated in agonist-induced depolarization of VSM (284). In smooth muscle, the estimated equilibrium potential for Cl^- (E_{Cl}) is somewhere between -47 and -10 mV (106), with the variation probably reflecting differences in the activity or expression of different Cl^- transport systems in different vessels. If E_{Cl} were more positive than the resting potential of the cell (3), opening of a Cl^--selective channel would allow Cl^- efflux, producing depolarization. Possible candidates mediating this effect would be a Ca^{2+}-activated Cl^- current (156, 221) and a volume-activated Cl^- current (387), both of which have been described in VSM.

In view of this, Nelson (264) has proposed that activation of Cl^- channels may explain stretch-induced depolarization of VSM. Support for this idea derives from the observation that Cl^- channel inhibitors (DIDS and indanyloxyacetic acid) hyperpolarize rat cerebral artery myocytes and inhibit myogenic tone of pressurized cerebral arteries (266). In addition, reduction of $[Cl^-]_o$ from ~120 to 60 mM (which shifts the calculated E_{Cl} to -2 mV) enhances pressure-induced myogenic tone in cerebral arteries.

Although this idea is intriguing, a subsequent and more thorough study casts doubt on these conclusions. Doughty et al. (74) tested the effects of Cl^- channel blockers on rat cerebral arteries using patch-clamp techniques in combination with isobaric and isometric measurements of mechanical activity. The Cl^- channel blockers flufenamic acid and 9-anthracine chloride, which are fairly specific for Ca^{2+}-activated Cl^- channels, had no effect on myogenic tone, even at high doses. Likewise, glibenclamide, an inhibitor of the cystic fibrosis transmembrane conductance regulator channel (as well as the K_{ATP} channel) was without effect on myogenic tone. 5-Nitro-2-(3-phenylpropylamino)benzoic acid, another Cl^- channel blocker, reversibly inhibited both myogenic tone and KCl-induced tone, but these effects were shown to be mediated by inhibition of VGC channels (74). At this time, the lack of specific blockers does not permit definitive conclusions to be made regarding the role of Cl^- channels in the myogenic response, but the existing evidence suggests they do not play an initiating role.

6. Voltage-gated Ca^{2+} channels

Voltage-gated Ca^{2+} channels have been recorded in many types of VSM, exhibiting characteristics of both L-type (15, 17, 101, 237, 269, 380) and T-type (15, 101, 230) channels. The L-type channel (also referred to as the VGC channel) is thought to be more important in arterial smooth muscle (267). In bath solutions containing physiological concentrations of Ca^{2+}, both the activation threshold (-50 to -60 mV) and peak current (-10 mV) for the L-type Ca^{2+} channel occur at negative potentials

(1). Because resting membrane potentials of VSM cells are in this range (132, 151, 262), a significant fraction of current must normally be activated at rest (98, 267, 305).

A large body of evidence now suggests that VGC channels play a central, obligatory role in determining myogenic responsiveness. *1*) Voltage dependence of the L-type channel predicts that the 20- to 35-mV depolarization associated with VSM stretch would increase the open probability of the VGC channel by 10- to 15-fold (267). *2*) Dihydropyridines eliminate or dramatically attenuate myogenic responsiveness in all (9, 132, 147, 170, 215, 369) but a few vessel types (159, 289) (the voltage dependence of dihydropyridine block may explain the discrepancies). *3*) Dihydropyridines attenuate pressure- or stretch-induced $[Ca^{2+}]_i$ increases in isolated arterioles (401) and VSM cells (64). *4*) Activators of VGC channels (e.g., BAY K 8644) enhance myogenic responses (83, 147, 202, 369). *5*) Elevated levels of $[Ca^{2+}]_o$ enhance both myogenic responsiveness and the degree of pressure-induced depolarization (132). This evidence does not rule out an upstream role for other types of channels that could regulate VGC channel gating by depolarization, but it indicates that Ca^{2+} influx through VGC channels is at least a common step downstream in the signaling pathway.

It should be pointed out that myogenic tone in a few vessel types, notably rabbit facial vein and ear artery, does not exhibit the same dependence on VGC channel-mediated Ca^{2+} entry as determined for other vessels (23). This has led to the conclusion that a unique Ca^{2+} entry pathway is activated by stretch (24, 379). The specific arguments for this are based on comparisons of stretch-dependent tone with KCl- and agonist-induced tone (the latter two presumably act through VGC channels). In facial vein, stretch-dependent tone *1*) has a different sensitivity to vasodilators, *2*) has a different sensitivity to Ca^{2+} channel blockers (379), *3*) is more susceptible to temperature changes (23), and *4*) is more susceptible to experimental trauma (23, 60). The reasons for these differences are not known, but it is possible that some vessel types rely more extensively on Ca^{2+} influx through MS cation channels than through VGC channels (also, the studies cited above were performed under isometric conditions).

There are at least three ways in which VGC channels might participate in myogenic responses: *1*) by opening when an upstream depolarizing stimulus brings the VGC channel to threshold (discussed in sect. IV*A3*), *2*) by a shift in the activation or inactivation curve of the VGC channel to a voltage range more favorable for opening, and *3*) by a direct effect of stretch on gating of the VGC channel.

With regard to the second mechanism, plots of open probability versus membrane potential for VGC channels show an activation threshold at approximately -50 mV and 90% inactivation at approximately -5 mV (values quoted for tracheal myocytes in bath solution containing

1.8 mM Ca^{2+}) (87). The relationship between the activation and inactivation curves predicts a voltage window (with a peak around ~30 mV) in which Ca^{2+} current can be sustained under physiological conditions (267, 305). This is confirmed by simultaneous measurements of $[Ca^{2+}]_i$ and current in voltage-clamped cells showing an excellent correlation between Ca^{2+} entry through VGC channels and depolarization-induced $[Ca^{2+}]_i$ increases (102, 191). Shifting the activation curve to more negative potentials would lead to increased VGC channel activation at rest, whereas shifting the inactivation curve to more positive potentials would result in less Ca^{2+}-induced inactivation and thus more sustained Ca^{2+} entry at any given potential. This effect is known to occur with some agonists and antagonists (1, 18) and may account for at least some of the potentiating action of α-adrenergic agonists on the myogenic response (160, 242).

In addition to the above mechanisms, VGC channels might be directly modulated by stretch. Current flow through VGC channels is unlikely to account for stretch-induced depolarization because the depolarization persists in the presence of Ca^{2+} channel blockade (205, 317). Also, estimates of channel density, cell size, and degree of steady-state inactivation make it unlikely that VGC channels contribute more than 2–5 pA of steady-state inward current at −40 mV (246). However, L-type Ca^{2+} currents in rat cerebral artery myocytes, as recorded using the conventional whole cell mode, are enhanced by inflating cells through the patch pipette (218, 239) and are enhanced in the perforated-patch recording mode by hyposmotic cell swelling (218). Similar findings have been reported in rabbit cardiac myocytes (238) and gastric myocytes (386). These results suggest L-type channels may be directly gated by membrane distension, although an alternative explanation is that cell volume changes following inflation or swelling lead to alterations in the concentration of intracellular second messengers that modulate channel activity (e.g., cAMP; Ref. 228). However, stretch-induced changes in L-type current occur whether or not ATP and GTP are added to the patch pipette (218), when a peptide inhibitor of cAMP-dependent protein kinase is present (238), and when intracellular Ca^{2+} is chelated with high concentrations of EGTA or BAPTA [1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid] to minimize Ca^{2+}-induced inactivation (238). Although the possible involvement of other second messenger systems [e.g., PKC (314), diacylglycerol (137, 352), and 20-HETE (136)] cannot be ruled out at this time, these experiments provide compelling evidence that VGC channels can be activated by VSM membrane stretch.

It remains to be determined if the degree of membrane stretch in inflation and swelling experiments corresponds to the force experienced by VSM cells in an intact vessel wall during physiological changes in pressure. In this regard, direct modulation of whole cell VGC channel

currents either could not be detected (58) or was reduced (due to Ca^{2+}-dependent inactivation) (363) in smooth muscle cells that were stretched longitudinally within apparent physiological limits. Also, neuronal VGC channels have been shown to be activated by flow (19), which would seem to be an unlikely physiological stimulus. It will be important to test if direct gating of VGC channels can be reproduced in single-channel recording modes and to determine the mechanosensitivity of the channel at that level; true MS channels typically change their open probability by three to four orders of magnitude following a mechanical stimulus (164), as opposed to more modest levels of mechanosensitivity that, under some circumstances, can be exhibited by agonist- and voltage-gated channels (285, 350).

B. Exchangers and Transporters

Vascular smooth muscle plasma membranes contain a number of carrier-mediated ion exchangers and transporters (273) whose function could potentially be modulated by membrane stretch. Active transport systems include a Na^+-K^+-ATPase, a Ca^{2+}-ATPase, and a K^+-H^+-ATPase; facilitated diffusion systems include Na^+/Ca^{2+} and Na^+/H^+ exchangers in addition to Na^+-Cl^--HCO_3^-, Na^+-K^+-$2Cl^-$, and Cl^--HCO_3^- cotransporters (273). Modulation of electrogenic pumps could directly stimulate membrane depolarization, whereas modulation of electroneutral exchangers could alter ion gradients; either of these mechanisms would impact myogenic tone if they altered Ca^{2+} availability to the contractile system.

The most likely candidates for relevant MS transporters would be the plasmalemmal Ca^{2+}-ATPase and the Na^+/Ca^{2+} exchanger. The Ca^{2+}-ATPase in VSM is regulated by calmodulin, by cGMP-dependent protein kinase, and possibly by phosphatidylinositol kinase (273), but no direct mechanical effects on this electrogenic pump have been reported. The Na^+/Ca^{2+} exchanger is regulated by PKC and by cGMP-dependent protein kinase, but again no direct effect of membrane stretch on this protein is known (273). In fact, no evidence points to direct MS regulatory control of any of the other transport systems in smooth muscle, with the possible exception of the Na^+-K^+-ATPase.

In VSM, the Na^+-K^+-ATPase is regulated by increases in $[Na^+]_i$, $[K^+]_o$, and by other ions including Ca^{2+}, Cd^{2+}, and vanadate. Protein kinase C and PKA also modulate Na^+-K^+ pump activity in smooth muscle (273). In cardiac myocytes, cell swelling induced by hypotonic solutions is associated with a 66% increase in Na^+-K^+-ATPase current (311), a response that is not secondary to accumulation of cytosolic Na^+, suggesting a direct mechanical effect. Although there is no electrophysiological evidence for MS Na^+-K^+ pump currents in smooth muscle, at least two

studies (142, 259) have examined the effects of cardiac glycosides such as ouabain (a Na^+-K^+-ATPase inhibitor) on myogenic tone. In rabbit facial vein, ouabain potentiates both myogenic- and agonist-induced tone, and a similar effect was recorded in cerebral artery strips using other cardiac glycosides (259). These results do not point to a role for the Na^+-K^+ pump in mediating the myogenic response but are consistent with the idea that the depolarizing action of ouabain (28) serves to enhance Ca^{2+} entry.

C. Enzyme Systems and Second Messengers

The lack of knowledge of the membrane system that transduces the mechanical stimulus imparted by pressure, along with difficulties in performing biochemical measurements on minute amounts of arteriolar smooth muscle, have hampered progress in understanding the intracellular signaling pathways involved in myogenic contraction. As a result, research efforts to date have typically used a framework based on studies of responses of VSM to contractile agonists in an attempt to understand the biochemical signals underlying the myogenic response. The following sections examine evidence for the involvement of a number of candidate intracellular signaling mechanisms in arteriolar myogenic responsiveness. Where data are available from studies of arterioles, these have been used. However, it remains necessary to extrapolate from studies of mechanical forces on other cell types and to consider data from conduit vessels and cultured cells.

1. Ca^{2+} as a second messenger

It has long been appreciated that Ca^{2+} plays a pivotal role in smooth muscle contraction and the setting of arteriolar tone (345). Removal of extracellular Ca^{2+} from isolated arterioles causes rapid relaxation and passive behavior. This effect of Ca^{2+} is assumed to be mediated through Ca^{2+}-calmodulin activation of myosin light chain kinase (MLCK) (Fig. 1). The following section examines the involvement of Ca^{2+} during myogenic vasoconstriction with an emphasis on sources of Ca^{2+}, modulation of Ca^{2+} sensitivity, and temporal aspects of signaling.

The importance of Ca^{2+} in arteriolar tone was first established by Uchida and Bohr (345) some 30 years ago. In this classic study, skeletal muscle small arteries developed an inherent level of tone that was abolished by perfusion with a Ca^{2+}-free solution. Dependence of single arterioles on an extracellular Ca^{2+} source for contraction and myogenic tone was first demonstrated by Duling et al. (75) in studies describing the isolated arteriole technique. Laher et al. (216) later demonstrated that myogenic tone shown by the rabbit facial vein was dependent on entry of Ca^{2+} as demonstrated by $^{45}Ca^{2+}$ influx. This technique,

however, lacks the sensitivity necessary for its application to single arterioles.

Although it is evident that arterioles possess functional intracellular Ca^{2+} stores (releasable by agonists, caffeine, and ryanodine), studies indicate that relative to conduit vessels, arterioles have a greater dependence on extracellular Ca^{2+} for contractile activity (42, 170). On the basis of studies of isolated intact hamster cheek pouch arterioles, this does not appear to reflect a fundamental difference in the sensitivity of the contractile proteins for Ca^{2+} (170) but may relate to factors such as *1*) smaller vessels having a relatively smaller volume of SR than larger vessels (10) or *2*) differences in the Ca^{2+} influx/efflux rates (170). In apparent contrast, Boels et al. (30) in studies of permeabilized mesenteric vessels have suggested that Ca^{2+} sensitivity of the contractile proteins is greater in arterioles than in conduit vessels.

The advent of Ca^{2+}-sensitive fluorescent dyes together with video-based imaging and photometer techniques have allowed the study of Ca^{2+} dynamics in true resistance vessels in a way that was not possible with radiolabeled tracer studies. Using isolated and cannulated skeletal muscle arterioles, Meininger et al. (243) first demonstrated that such approaches could be used to define arteriolar smooth muscle intracellular Ca^{2+} signaling during agonist and myogenic stimulation. Care was taken to exclude significant involvement of the endothelium by loading of the Ca^{2+}-sensitive dye from the abluminal surface, focusing on the outer cell layers of the vessel and demonstrating similar results in the presence and absence of a functional endothelial layer. An acute pressure step equivalent to 40 cmH$_2$O resulted in an increase in $[Ca^{2+}]_i$ of ~15% above baseline. The initial increase in Ca^{2+} appeared to parallel the pressure-induced distension of the vessel. In addition to examining changes in $[Ca^{2+}]_i$ during myogenic constriction, responses were also examined after stimulation of the arterioles with either NE or the PKC activator indolactam. Despite these agents causing a similar level of constriction, the adrenergic response occurred in the presence of a large increase in $[Ca^{2+}]_i$, whereas the PKC-mediated response occurred without a change in $[Ca^{2+}]_i$. These data therefore not only demonstrated pressure-induced increases in arteriolar wall $[Ca^{2+}]_i$ but also suggested that arterioles, like conduit vessels, possess mechanisms for modulating Ca^{2+} sensitivity (see also sects. *ivC1*c and *ivC4*). These basic results have been confirmed in a number of subsequent studies (50, 206, 348, 401).

Given the biphasic nature of the mechanical response of an arteriole to an increase in intraluminal pressure, it is clearly important to consider temporal aspects of $[Ca^{2+}]_i$ signaling if the role of this cation is to be understood. The schematic diagram shown in Figure 6 depicts the temporal aspects of the diameter response of
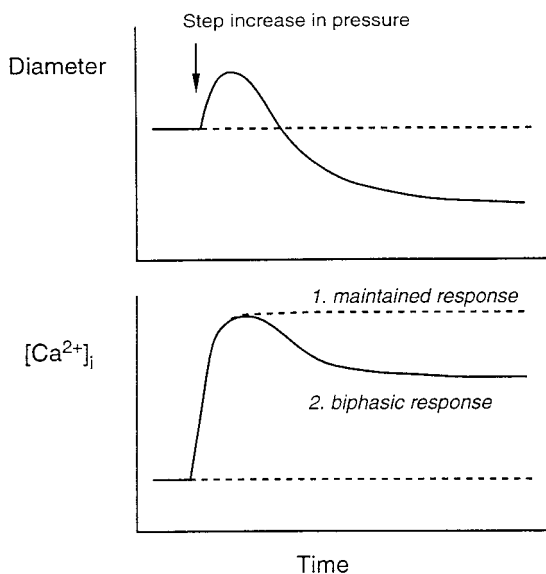
FIG. 6. Temporal pattern of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) changes in arteriolar vascular smooth muscle in response to a step elevation in transmural pressure (for details, see text).

an arteriole to an acute increase in intraluminal pressure together with possible intracellular Ca^{2+} signals.

Figure 6 illustrates that an arteriole passively distends in response to an acute pressure increase, followed by a constriction to a steady-state diameter that is typically smaller than that before the pressure step. This mechanical response has been shown to be associated with either *1*) a monophasic increase in $[Ca^{2+}]_i$ where Ca^{2+} peaks following distension and remains at that level for the duration of the pressure increase or *2*) a biphasic increase in $[Ca^{2+}]_i$ where an initial peak is followed by a decline to a steady-state $[Ca^{2+}]_i$ level that remains elevated relative to baseline. Although both $[Ca^{2+}]_i$ patterns have been reported, there are several explanations for this apparent inconsistency. First, the magnitude of the initial peak in $[Ca^{2+}]_i$ appears to be related to the extent of the pressure-induced distension, possibly reflecting a change in cell length or wall tension. Thus larger pressure steps may amplify the appearance of a biphasic pattern. The magnitude of the change in $[Ca^{2+}]_i$ associated with a pressure increase is small relative to that seen with agonists, so the study of $[Ca^{2+}]_i$ responses to small pressure steps is therefore more dependent on the sensitivity of the measurement techniques. As such, it may be difficult to resolve a biphasic $[Ca^{2+}]_i$ change in arterioles exposed to relatively small changes in pressure. A further consideration is that as the vessel constricts, reflecting shortening of the smooth muscle cells, the stimulus for Ca^{2+} mobilization presumably decreases; a biphasic $[Ca^{2+}]_i$ pattern might, therefore, be expected. However, it could be argued that this would be predicted by any model of Ca^{2+} availability and is not necessarily an indication of tempo-

ral variation in the contribution of Ca^{2+} pools or the participation of alternate regulatory mechanisms/Ca^{2+} sensitization in the steady state. An additional explanation for the biphasic change in $[Ca^{2+}]_i$ relates to the possibility that the initial increase in $[Ca^{2+}]_i$ activates an inhibitory process aimed at dampening the $[Ca^{2+}]_i$ rise and hence vasoconstriction (265). Again, such a process may be expected to be more evident following large pressure steps that are associated with relatively larger $[Ca^{2+}]_i$ peaks.

The above discussion has not considered whether the distension-induced $[Ca^{2+}]_i$ peak and the steady-state $[Ca^{2+}]_i$ level are related or the relative roles of these phases in the contractile response. In an effort to determine if the initial increase in $[Ca^{2+}]_i$ was necessary to elicit steady-state myogenic contraction, the responses of isolated arterioles to 30- to 120-mmHg pressure increases were compared when the pressure change was delivered either instantaneously or as a ramp function over 5 min (148). During the latter protocol, the rapid pressure-induced distension and the associated transient increase in $[Ca^{2+}]_i$ is avoided. Despite this, the steady-state diameter achieved is similar under both protocols (64), suggesting that the initial peak in $[Ca^{2+}]_i$ is not an absolute requirement for effective myogenic constriction. Similarly, D'Angelo et al. (50), in a study of isolated hamster cheek pouch arterioles, demonstrated that although the degree of distension was related to the peak change in $[Ca^{2+}]_i$, steady-state $[Ca^{2+}]_i$ levels were similar regardless of the size of the applied pressure step. Steady-state constriction was greater, however, in vessels exposed to larger pressure steps. It was suggested that an excess of Ca^{2+} (relative to a required threshold level) was mobilized during the initial phase and that processes of Ca^{2+} sensitization were activated during the maintained or steady-state phase. With the consideration of both sets of data, however, it could be argued that more than one event occurs, for example, a purely mechanical or stretch-mediated response that occurs with distension and a second phase related to a variable other than overt cell length, such as wall tension. Interestingly, when cannulated skeletal muscle arterioles were subjected to acute longitudinal stretch, the vessels responded with a rapid increase in $[Ca^{2+}]_i$ which then returned to baseline levels despite maintenance of the stretch stimulus (S. Potocnik, M. J. Davis, H. Zou, S. Price, and M. A. Hill, unpublished observations).

A) INTERRELATIONSHIPS BETWEEN Ca^{2+} SOURCES. The involvement and relative roles of specific Ca^{2+} sources in the myogenic response still remain uncertain. Although it is clear that there is a major dependency on extracellular Ca^{2+}, questions remain as to the specific entry mechanisms (see sect. ${\rm IV}A6$) and the involvement of release from compartments such as the SR. It is apparent that arteriolar smooth muscle possesses sarcoplasmic Ca^{2+} stores released by activation of either inositol trisphosphate

(IP$_3$) or ryanodine receptors, with the latter being involved in Ca^{2+}-induced Ca^{2+} release (192, 265). The involvement of IP$_3$-mediated Ca^{2+} release from the SR is supported by studies demonstrating accumulation of inositol phosphates following length or pressure changes (143, 261, 391). These changes are not necessarily associated with myogenic behavior (391). Furthermore, when briefly exposed to 0 mM extracellular Ca^{2+} solutions, arterioles respond to agonist stimulation with transient contractions consistent with the presence of a releasable intracellular Ca^{2+} store. This store appears to be more rapidly depleted than in larger vessels exposed to similar agonist stimulation (170).

There is little evidence to suggest that myogenic vasoconstriction depends on the release of intracellular Ca^{2+}. Nakayama et al. (260) reported that depletion of SR Ca^{2+} with either ryanodine or dantrolene led to inhibition of stretch-induced tone in rabbit cerebral artery strips. In contrast, other studies performed in the presence of ryanodine have shown that isolated, cannulated arterioles retain myogenic responsiveness (239, 358, 401), although in two of those, it was reported that this agent decreases the rate of onset of the mechanical response (358, 401). Watanabe et al. (359) further reported that ryanodine and the SR Ca^{2+}-ATPase inhibitor cyclopiazonic acid enhanced arteriolar tone in myogenically active vessels. This latter result can be explained by possible removal of a Ca^{2+} buffering action of the SR. These apparent differences may relate to the vessels or techniques (arterial strips versus cannulated arterioles) that were used. Alternatively, there may be a different dependence on Ca^{2+} pools for responses of tissues undergoing acute stretch as compared with an increase in transmural pressure. That stretch of smooth muscle cells mobilizes intracellular Ca^{2+} was demonstrated by the studies of Davis et al. (62), where acute stretch of single coronary artery smooth muscle cells, bathed in a Ca^{2+}-free solution, resulted in the release of intracellular Ca^{2+}, presumably from the SR. Collectively, these studies suggest that although smooth muscle SR Ca^{2+} is released in response to mechanical stimuli, it is not critical to myogenic constriction; rather, the release of intracellular Ca^{2+} may play a role in the onset of contraction or, alternatively, may be involved in regulatory mechanisms involved in Ca^{2+} entry or in limiting the extent of contraction (see below).

Recently, attention has been given to the role of spatial aspects of Ca^{2+} signaling during arteriolar myogenic activation. Although the studies referred to above using fluorescent indicator techniques have only considered global smooth muscle [Ca^{2+}]$_i$, it has been suggested that Ca^{2+} released within microdomains may serve a regulatory role other than direct activation of contraction through the Ca^{2+}/calmodulin/MLCK pathway. Nelson et al. (265) have suggested that release of Ca^{2+} from the SR, by way of a ryanodine-sensitive mechanism, acts not to cause an increase in global cytosolic Ca^{2+} but to directly activate K$_{Ca}$ channels. Thus this microdomain of Ca^{2+} provides a hyperpolarizing stimulus that acts as a negative feedback, by inhibition of VGC channels, to inhibit contraction, an action somewhat counterintuitive to the role of global [Ca^{2+}]$_i$ in contraction per se. Support for such a release of Ca^{2+} within microdomains, or Ca^{2+} sparks, also comes from studies in other tissues, such as cardiac myocytes (371).

The presence of a functional SR in arterioles and evidence suggesting that pressure leads to mobilization of intracellular Ca^{2+} leads to questions regarding the possible role of two other models for spatial Ca^{2+} signaling, namely, the superficial buffer-barrier hypothesis (41, 263) and the relationship between Ca^{2+} entry and refilling of the SR Ca^{2+} store (store depletion-mediated Ca^{2+} influx). These models are schematically depicted in Figure 7. Although the models have not been thoroughly examined in isolated arterioles and their role in myogenic responsiveness remains uncertain, they are briefly described. The buffer-barrier hypothesis suggests that during Ca^{2+} entry a fraction of the Ca^{2+} influx is taken directly into the SR and is not made available for myosin light chain (MLC)-mediated constriction. As the SR fills with Ca^{2+}, there is leak of the cation into the submembranous space where it is extruded from the cell by mechanisms such as Na$^+$/Ca^{2+} exchange. This mechanism therefore acts to decrease, or buffer, a rise in cytoplasmic Ca^{2+}. In contrast, the store depletion-mediated Ca^{2+} influx model suggests that depletion of the intracellular store, after contractile activation, stimulates Ca^{2+} entry from the extracellular space (for review, see Refs. 22, 286). Because there appears to be no direct contact between the SR and the plasma membrane (263), this implies a role for either *1*) an as yet unknown factor that relays the filling state of the SR to a Ca^{2+} entry mechanism or *2*) conformational coupling, a process by which depletion of the store changes the conformation of an endoplasmic reticulum (ER) membrane protein allowing interaction with the Ca^{2+} entry channel (22). Although this model relating Ca^{2+} entry to the filling state of the ER was first demonstrated in Jurkat (295) and mast (161) cells, there is evidence for its existence in vascular cells including aortic smooth muscle (32, 392), smooth muscle cell lines (A7r5 cells; Refs. 27, 326), and endothelium (63).

Although not directly examining these mechanisms, Knott et al. (207) have suggested that their studies of Ca^{2+} signaling in pressurized cerebral arteries are not consistent with a role for either of these mechanisms. Furthermore, Skutella and Rüegg (326) have shown that depolarization inhibits store depletion-mediated Ca^{2+} influx (by thapsigargin). This effect of depolarization was independent of dihydropyridine-sensitive Ca^{2+} channels and was therefore suggested to be a function of the altered electrochemical gradient. Thus, if myogenic constriction re-
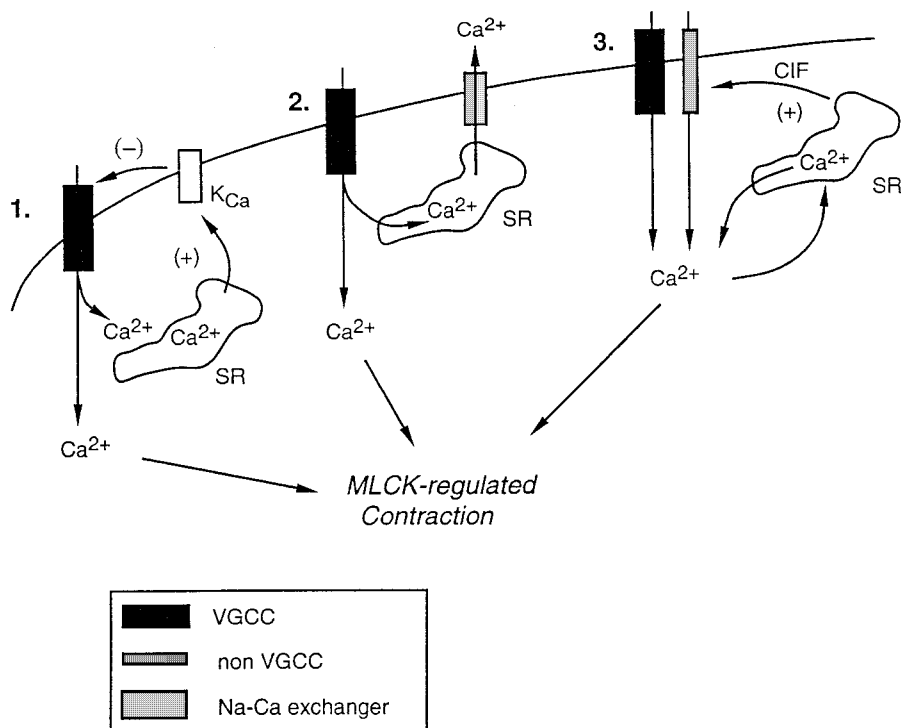
FIG. 7. Possible spatial aspects of vascular smooth muscle Ca^{2+} signaling in arterioles: *1*) Ca^{2+} sparks and activation of Ca^{2+}-activated K^+ channels (K_{Ca}); *2*) superficial buffer barrier hypothesis; and *3*) store depletion-mediated Ca^{2+} influx. For details, see text. MLCK, myosin light-chain kinase; SR, sarcoplasmic reticulum; VGCC, voltage-gated Ca^{2+} channel.

sults from depolarization-enhanced Ca^{2+} entry through VGC channels, then this may argue against involvement of store depletion-mediated Ca^{2+} entry. Because data are currently limited, it will be important in terms of understanding the dynamics of Ca^{2+} signaling, and the relative roles of intracellular and extracellular Ca^{2+} sources during myogenic constriction, to determine the importance of such mechanisms in intact arteriolar smooth muscle.

B) CA^{2+} SIGNALING BETWEEN CELLS. In addition to movement of Ca^{2+} from the extracellular space into the cytosol, the presence of communicating channels, such as gap junctions, may allow the movement of small signaling molecules between cells of the vascular wall. Studies by Duling and colleagues (124, 226, 384) have indicated that connexins [for example, connexin (Cx)40 and Cx43] exist between VSM cells, between endothelial cells, and to a lesser extent between VSM and endothelial cells. Such pathways are thought to provide direct electrical coupling along the vessel wall and allow the propagation of vasomotor activity. With respect to the myogenic response, Rivers (302) has recently demonstrated the propagation of pressure-mediated vasoconstriction. Although that study did not specifically investigate the nature of the signal transferred between cells, it has been shown that Ca^{2+} can pass through gap junctions (65). The significance of this phenomenon is at present unclear because it would appear to be too slow to account for a conducted response (73). However, it could conceivably contribute to the spread of a myogenic response along a single vessel or allow for coordination of responses between consecutive branching orders of an arteriolar network.

An important aspect of gap junction physiology that may relate to, or be affected by, a given level of myogenic tone is the acute regulation of channel-gating properties. Recent studies have shown that the permeability of gap junctions can be acutely modulated by second messenger-mediated mechanisms involving Ca^{2+}, cyclic nucleotides, or protein phosphorylation (for review, see Ref. 65). As such, if the level of myogenic activation determines the availability of second messengers, conductance of these intercellular channels may influence the ultimate level of vascular tone. The acute interaction between myogenic tone and gap junction conductance may also underlie recent controversy relating to the relative importance of homocellular (VSM to VSM or endothelial cell to endothelial cell) versus heterocellular (VSM to endothelial cell) coupling within the arteriolar wall (72, 365, 366, 383, 384).

C) MODULATION OF CA^{2+} SENSITIVITY. Although the fundamental biochemical process underlying smooth muscle contraction involves Ca^{2+}/calmodulin/MLCK regulation of actin-myosin interaction (see sect. IV*C2*), it is evident that there is not an invariant relationship between $[Ca^{2+}]_i$ and a given level of contraction (252). It has been shown that mechanisms exist by which the $[Ca^{2+}]_i$-contraction relationship can be shifted to the left (indicative of sensitization; Refs. 203, 272) or shifted to the right (desensitization) (149). The process of sensitization has been best demonstrated for receptor-mediated agonist responses

and may involve intracellular signaling mechanisms that utilize small-molecular-weight G proteins (312), PKC (236), and arachidonic acid (99, 109). Reference to such mechanisms can be found in the individual sections dealing with these mediators.

Although it is evident that resistance vessel smooth muscle possesses mechanisms for altering Ca^{2+} sensitivity (107, 145), it remains controversial as to whether or not the level of intravascular pressure can specifically lead to an alteration in the arteriolar smooth muscle $[Ca^{2+}]_i$-contraction relationship. Studies of the basic $[Ca^{2+}]_i$-contraction relationship are made difficult by the fact that permeabilization of arteriolar smooth muscle necessarily negates the membrane potential and therefore inactivates a critical signal transduction mechanism. By analogy to other muscle types, however, it could be argued that stretch may impart an effect on the contractile proteins through a length-dependent mechanism or, alternatively, by direct activation of a biochemical mechanism that modulates Ca^{2+} sensitivity.

McCarron et al. (239) recently examined the effect of intraluminal pressure on the $[Ca^{2+}]_i$-contraction relationship of α-toxin-permeabilized cerebral arterioles. Despite examining vessels under Ca^{2+}-clamped conditions over the range 1 nM to 60 μM, no evidence was found to support pressure-induced modulation of Ca^{2+} sensitivity. Under the conditions of that experiment, arterioles responded passively to increases in intraluminal pressure regardless of the intracellular Ca^{2+} concentration. It is, however, conceivable that Ca^{2+} sensitization requires both an intact membrane system and events distal to this point. In contrast, VanBavel et al. (348) have recently reported that cannulated small mesenteric arteries do show Ca^{2+} sensitization at increased pressures. It was concluded that electromechanical coupling alone could not explain myogenic responsiveness because the slope of the $[Ca^{2+}]_i$-tone relationship (defined as the ratio of active wall tension to maximally active wall tension) was five times greater for pressure activation as compared with that for KCl. This study is one of the few to separate the effects of length from activation in small vessels, but interpretation is complicated by the fact that the preparation possessed little inherent myogenic tone and in many cases required preactivation with NE. Although the authors indicated that apparent constrictor responses were similar in vessels either preactivated or studied under conditions of spontaneous tone, the rationale for using a receptor-mediated agent that is known to stimulate pathways leading to Ca^{2+} sensitization must be questioned. Thus, at this point, it appears premature to definitely state whether or not myogenic activation is associated with an increase in Ca^{2+} sensitivity and, furthermore, if sensitization does occur, the mechanisms involved have yet to be identified.

2. Control of MLC phosphorylation

Any step in the activation sequence, from control of $[Ca^{2+}]_i$ to contractile protein Ca^{2+} sensitivity, might conceivably depend on muscle length. In cardiac muscle, the steepness of the steady-state length-tension relationship can be explained primarily by changes in Ca^{2+} sensitivity, whereas the slow changes in tension developed after an alteration in length involve changes in both Ca^{2+} supply and Ca^{2+} sensitivity (5). Changes in Ca^{2+} sensitivity are not explained by changes in the affinity of troponin C for Ca^{2+} (357), which is consistent with the persistence of this phenomenon in smooth muscle. At the present time, it is unclear how many of the mechanisms in cardiac muscle (or even skeletal muscle) are relevant to smooth muscle.

Although it is generally accepted that myogenic contractions of arterioles occur by the classical mechanism involving Ca^{2+}/calmodulin-dependent phosphorylation of the 20-kDa myosin regulatory light chains (Fig. 8; for review of smooth muscle contraction, see Refs. 158, 258, 329), there are comparatively little data directly validating this assumption. This is due in large part to technical difficulties associated with performing phosphorylation measurements on small tissue samples. As a result of this, the involvement of this regulatory mechanism in myogenic constriction has largely been inferred on the basis of studies performed by Barany and co-workers (12, 13, 222) using carotid artery strips. In their initial study in 1983, Ledvora et al. (222) showed that acute stretch to 1.7 times the resting length resulted in increased MLC phosphorylation from 33% at rest to 56% after application of the mechanical stimulus. In later studies, these observations were extended to show that the extent of phosphorylation was directly proportional to the degree of applied stretch and that the phosphorylated sites (as determined by phosphopeptide mapping) on the myosin regulatory light chains were consistent with the action of MLCK as opposed to another kinase such as PKC (12, 13). A number of studies in vascular (126, 240, 300) and nonvascular (391) smooth muscle have demonstrated length dependence of MLC phosphorylation. Such preparations, however, would not be expected to exhibit myogenic contraction, and furthermore, the degree of stretch was often greater than would be expected in an arteriole exposed to a physiological pressure change. An additional distinguishing feature between these studies of conduit arteries and arterioles is that stretch-induced phosphorylation was dependent on intracellular Ca^{2+} release, whereas myogenic contraction in arterioles occurred largely through a mechanism involving Ca^{2+} entry from the extracellular space.

To examine relationships between MLC phosphorylation and peripheral arterial resistance, Moreland et al. (251) collected samples of canine anterior tibial artery
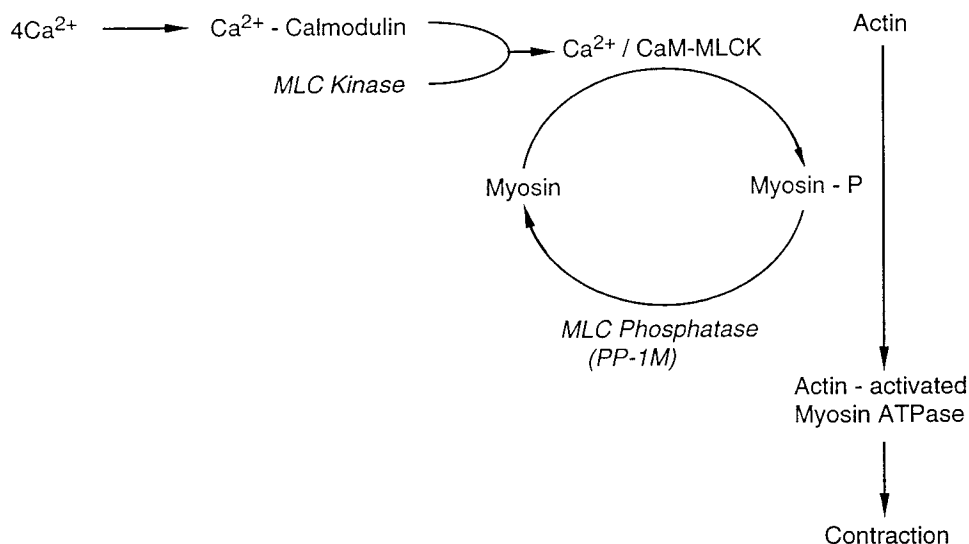
$4Ca^{2+} \longrightarrow Ca^{2+}$ - Calmodulin

MLC Kinase

Ca^{2+} / CaM-MLCK

Actin

Myosin

Myosin - P

MLC Phosphatase (PP-1M)

Actin - activated Myosin ATPase

Contraction

FIG. 8. Thick-filament regulatory pathway for smooth muscle contraction, involving Ca^{2+}, calmodulin (CaM), and myosin light-chain kinase (MLCK) (for details, see text).

under blood-perfused in vivo conditions. Tissue samples were rapidly frozen and subjected to two-dimensional gel electrophoresis. These authors reported an increase in the level of phosphorylation following superfusion with the contractile agonist phenylephrine. Although an in vivo preparation was used, the particular vessel collected would not have been expected to possess a significant level of inherent basal tone. Consistent with this, a relatively low level of phosphorylation ($13 \pm 2\%$) was obtained in the absence of the exogenous adrenergic stimulus; this value is similar to that typically obtained at baseline in conduit vessels studied under in vitro conditions (e.g., Ref. 298). Using a ring preparation of rabbit facial vein, LaPorte et al. (220) reported that stretch-induced tone was associated with an increased level of MLC phosphorylation. It is unclear whether this specialized venous preparation is, however, representative of arteriolar smooth muscle.

More recently, two-dimensional gel electrophoresis methods have been developed to the point where it has been possible to measure MLC phosphorylation on pooled samples of in vitro pressurized arterioles. Using cannulated rat cremaster muscle first-order arterioles, Zou et al. (400) demonstrated that the steady-state level of MLC phosphorylation increased with increasing intraluminal pressure. Interestingly, the arterioles were found to maintain a relatively high steady-state level of phosphorylation ($>25\%$), relative to that for conduit vessels ($\sim14\%$), indicating that they remain in an activated state after achieving a stable myogenic contraction. Inhibition of myogenic reactivity and phosphorylation by either removal of extracellular Ca^{2+} or treatment with the MLCK inhibitor ML-9 suggested that pressure-induced phosphorylation occurred by way of the classical Ca^{2+}/calmodulin/MLCK-mediated pathway. In apparent contrast to these findings, Pawlowski and Morgan (289) found that ferret

aorta is capable of demonstrating an intrinsic, temperature-sensitive tone that could not be completely explained by a mechanism involving MLC phosphorylation. As with the work of Barany et al. (12), it is not clear whether these studies are directly relevant to arterioles; a possible difference between the vessel types is evident by the observation that intrinsic tone of the ferret aorta preparation occurred with a MLC phosphorylation level of $\sim11\%$, whereas data for arterioles with spontaneous tone indicate a phosphorylation level of $>25\%$ is required to maintain myogenic constriction (400).

The finding that MLC phosphorylation was obligatory for the expression of myogenic reactivity does not, however, rule out an involvement of other systems regulating contraction at the level of the contractile proteins. Given the data accumulating with respect to agonist contractions, it is conceivable that additional regulatory mechanisms act in parallel to MLC phosphorylation (see Ref. 158). For example, in permeabilized small mesenteric arteries, it was shown that the PKC activator indolactam caused constriction by a mechanism involving an increase in the level of MLC phosphorylation, but in the absence of an increase in Ca^{2+} above resting levels (145). Such a mechanism would be consistent with Ca^{2+} sensitization mechanisms involving inhibition of MLC phosphatase that has been described for conduit vessels (329). Despite the likelihood that these mechanisms exist in resistance vessel smooth muscle, whether they are invoked during myogenic activation is at present controversial. For example, in recent experiments using isolated cerebral arterioles, McCarron et al. (239) found that myogenic tone was dependent on voltage-gated Ca^{2+} entry (although evidence for modulation of this mode of Ca^{2+} entry was apparent) and not on Ca^{2+} sensitization. Likewise, Zou et al. (401) have reported that cremaster muscle arteriolar smooth muscle contraction to adrenergic agonists in-

volves both modulation of Ca^{2+} sensitivity and the classical MLC phosphorylation pathway, whereas myogenic contraction is totally dependent on voltage-gated Ca^{2+} entry and subsequent activation of MLCK. In contrast, VanBavel et al. (348; see also sect. IV*C1*), in studies of small mesenteric arteries, have suggested that during myogenic contraction the $[Ca^{2+}]_i$-tone relationship is too steep to be explained by electromechanical coupling and that Ca^{2+} sensitization mechanisms must be involved. Inhibition of voltage-gated Ca^{2+} entry with nifedipine did, however, abolish the myogenic response, indicating an obligatory role of this pathway. Further studies are required to determine whether Ca^{2+}-mediated MLC phosphorylation is sufficient to explain myogenic contraction or whether such results are explained by tissue-specific differences (see also sect. IV*C1*).

There are very few data examining temporal relationships between $[Ca^{2+}]_i$, MLC phosphorylation, and arteriolar diameter during a myogenic contraction. Drawing again on studies of conduit arteries, Barany et al. (12) have suggested that although MLC phosphorylation is required for activation of smooth muscle following stretch, it is not an absolute requirement for maintenance of stretch-induced tension. This suggestion was based on the observation that when carotid artery strips stretched to 1.7 times resting length are released, tension develops while MLC phosphorylation decreases. In addition, La-Porte et al. (220) found that activation of PKC potentiates steady-state myogenic tone in rabbit facial vein in a manner not requiring an increase in $[Ca^{2+}]_i$ or MLC phosphorylation. Both groups of investigators speculated that mechanisms other than MLC phosphorylation are involved in myogenic contraction, particularly during the sustained phase of the mechanical response. This may be unique to isometric preparations, because data obtained in studies of cannulated arterioles (diameter $\sim100~\mu$m) do not support an obligatory role for mechanisms other than MLC phosphorylation in the sustained phase of a myogenic response (400-402). As mentioned above, the MLCK inhibitors ML-7 and ML-9 inhibit sustained arteriolar myogenic tone while also decreasing the level of phosphorylation occurring during the response to an acute pressure step. In addition, the temporal pattern of MLC phosphorylation during a myogenic response appears to be largely monophasic (401) as compared with the response to agonists that is often biphasic: an initial increase in phosphorylation is followed by a decline to a plateau during the sustained phase of contraction. In the case of agonist contraction, the dissociation between steady-state force maintenance and levels of MLC phosphorylation have been considered to be consistent with either Ca^{2+} sensitization or the existence of alternate regulatory mechanisms.

3. Mechanisms other than MLC phosphorylation

The specific involvement of other smooth muscle actin and myosin binding proteins (for example, caldesmon and calponin) in myogenic contraction has not been investigated. However, modulation of their actin/myosin binding functions, together with inhibition of myosin ATPase (140, 377) through phosphorylation mechanisms, makes them attractive candidates for a role in the sustained phase of a myogenic response. In addition, no data exist in arteriolar smooth muscle as to mechanisms by which MLCK is itself regulated via phosphorylation. In large vessels and nonvascular smooth muscle, MLCK is desensitized by Ca^{2+}/calmodulin-dependent protein kinase II, thus providing a mechanism for modulating contraction (172).

It is well established that contractile proteins exist as isoforms and that there appears to be variation in the expression of a given isoform between species and tissues. Thus there are at least three isoforms of actin (α, β, γ) and multiple forms of both the myosin heavy and light chains (85, 139, 304). Knowledge of the relative content and distribution of potential thin filament regulatory proteins (e.g., caldesmon and calponin) and their isoforms in arteriolar smooth muscle is totally lacking. It is, however, intriguing to speculate that differences in the contractile protein or isoform complement could be involved in differences in function between large and small arteries, including myogenic properties. In support of this suggestion, DiSanto et al. (70), using RT-PCR, have demonstrated differences in the mRNA coding for myosin heavy chains of aorta as compared with small muscular arteries. Furthermore, aorta was shown to have approximately equal amounts of the a- and b-17-kDa essential light chain isoforms (LC17), whereas the smaller arteries had predominately the a-isoform. Studies performed in other smooth muscles suggest that the relative composition of the LC17 isoforms may impact on muscle contractile function as reflected by differences in maximum velocity or phasic versus tonic behavior (235, 340). These observations, together with a greater inherent ATPase activity in the smaller arteries, have led investigators to suggest that differences in the contractile characteristics of small and large arteries may be a function of the expressed myosin isoforms. The extent to which contractile protein isoform may determine myogenic behavior is unknown at this time. The availability of techniques such as RT-PCR, together with sensitive electrophoretic methods and confocal microscopy, should soon allow these analyses to be conducted on true resistance vessels.

4. PKC

Interest in a possible role for the serine/threonine kinase PKC in the myogenic response has followed from studies demonstrating that agonists such as NE enhance

myogenic responsiveness (83, 242, 299, 334, 335, 347). These agonists work in part through activation of PKC. Subsequent studies showed that more specific activators of PKC (e.g., phorbol esters, indolactam) are potent vasoconstrictors and that PKC activation is associated with enhanced contractile protein Ca^{2+} sensitivity (43, 297). Although the exact mechanisms by which PKC activation leads to contraction are uncertain, it is apparent that it does not directly increase the level of Ser-19 phosphorylation of the regulatory myosin light chains. Biochemical studies indicate that PKC-mediated phosphorylations of both 20-kDa light chain and MLCK are, in fact, inhibitory on actomyosin ATPase activity (16, 339). Two-dimensional gel electrophoresis has been used to show that the PKC-mediated protein phosphorylation pattern resembles that occurring during the maintained phase of an agonist-induced contraction rather than that which can simply be attributed to MLCK-induced phosphorylation (297). Because PKC has been implicated in the maintained phase of agonist-induced contractions, it has made intuitive sense that this enzyme may play a role in tonic myogenic contractions. Furthermore, if a myogenic stimulus activates membrane-bound phospholipases (in particular PLC), then diacylglycerol, the endogenous activator of many of the PKC isozymes, would be formed along with IP_3.

Initial studies implicating a role for PKC in myogenic signaling utilized small-molecular-weight inhibitors such as 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7), staurosporine, and calphostin C (146, 214, 281). These inhibitors, however, tend to suffer from a lack of specificity, and as a result, data from such studies must be interpreted with caution. For example, both H-7 and staurosporine, although being structurally dissimilar, compete at the ATP binding site, a site that is highly conserved between the various protein kinases (306). Thus there is potential for nonspecific inhibition of MLCK and misinterpretation of results. With this caveat in mind, it should be appreciated that the more specific but larger molecular weight peptide inhibitors (for example, those aimed at the pseudosubstrate region of the enzyme) require that smooth muscle cells be permeabilized. As mentioned previously, this procedure negates the membrane potential and interferes with ionic mechanisms integral to the membrane components of the myogenic transduction mechanism. An important future direction will be the development of methods to deliver such specific inhibitors into cells of fully functional arterioles with intact electrophysiological mechanisms.

Using isolated and cannulated rat cerebral arterioles, Osol et al. (281) showed dose-dependent inhibition of spontaneous myogenic tone by staurosporine, implicating a role for PKC. In rat cremaster muscle arterioles studied under in vivo conditions, staurosporine inhibited the constrictor response to an acute increase in arteriolar pressure; however, it had little effect on baseline diameter

(146). The reasons underlying the differences between these studies are uncertain but may relate to *1*) the differing vessel sizes examined (150 versus 20 μm); *2*) the different tissues (cerebral versus skeletal muscle) from which the vessels were obtained; or *3*) the fact that one study was performed in vitro and nonperfused while the other was performed under in vivo, blood-perfused conditions. Regardless of the differences, these results are consistent with a possible role for PKC in myogenic signaling. More recently, a role for PKC in myogenic tone has also been demonstrated in studies of isolated human coronary arterioles (247). These findings are also supported by studies of rabbit facial vein (214) and ferret aorta (289) where staurosporine was shown to inhibit intrinsic tone. In addition to studies utilizing inhibitors, support for a role for PKC in myogenic signaling has been provided by a number of investigators who have shown that pharmacological activators of PKC (146, 281), or subcontractile concentrations of receptor-mediated agonists (202, 232), enhance arteriolar myogenic reactivity.

Additional supporting evidence for a role for PKC in myogenic signaling was provided by the observation that diacylglycerol accumulates in cannulated renal arcuate arteries following an increase in intraluminal pressure (261). Diacylglycerol is required in the physiological activation of a number of the PKC isozymes. From the temporal data provided in that study, it is difficult to determine with any certainty whether the activation of PKC in renal vessels would be consistent with a role in contractile function or whether activation of the kinase may be a reflection of other cellular processes initiated by the mechanical stimulus, such as a growth response.

How PKC participates in myogenic reactivity remains uncertain; however, because PKC activators have been shown to increase the level of arteriolar tone without an overt increase in $[Ca^{2+}]_i$ levels, it is tempting to speculate that the kinase exerts an effect through modulation of Ca^{2+} sensitivity. In a recent study, Karibe et al. (194) have suggested that both a rise in $[Ca^{2+}]_i$ and an increase in PKC activity are required for full myogenic contraction. These authors speculate that PKC is involved in coupling the $[Ca^{2+}]_i$ increase to the contractile process by some yet-to-be-defined mechanism. Caution must be exercised in interpreting these data, however, because that study did not include measurements of PKC activity, and kinase inhibitors were used without assessing nonspecific effects on contraction or the level of MLC phosphorylation.

A distinct possibility is that effects of PKC are mediated through a parallel mechanism that affects contraction per se rather than being fundamentally involved in the myogenic signaling pathway itself. For example, PKC has been implicated as both an inhibitor (100) and an activator (86, 145) of VGC channels in various preparations; obviously by modulating Ca^{2+} availability PKC could influence the extent of myogenic reactivity. In re-

cent studies of afferent arterioles, Kirton and Loutzenhiser (202) have suggested that PKC modulates the activity of K_v channels (48) that would indirectly potentiate myogenic contraction. This was demonstrated by the finding that the effects of PKC inhibition on attenuation of myogenic reactivity could be reversed by treatment with 4-aminopyridine, a known inhibitor of K_v channels. In addition to simply acting on parallel pathways, PKC activation can possibly lead to multiple effects in one tissue; thus it has been reported that the PKC activator indolactam both enhances Ca^{2+} current through VGC channels and enhances Ca^{2+} sensitivity at the level of the contractile proteins (145).

Studies of the involvement of PKC in myogenic signaling mechanisms have been made difficult by *1*) the fact that there are at least 11 different isozymes of PKC (155), with multiple forms existing within a given cell type; *2*) lack of specificity of inhibitors that can be used in intact systems; *3*) a lack of information as to the specific substrates of the enzyme that are phosphorylated during myogenic activation; and *4*) measurements of PKC activity and translocation have not been performed during arteriolar myogenic responses. As specific and isozyme-directed inhibitors are developed (for example, LY-333531, an inhibitor of the PKC β-isoform; Ref. 39), the role of PKC in arteriolar myogenic responses may be elucidated.

5. *G proteins, PLC, and phosphoinositide metabolism*

The finding that plasma membrane receptor activation by agonists typically leads to G protein-mediated stimulation of a phosphatidylinositol-specific PLC and production of IP_3 has stimulated interest in the possible role of this pathway in arteriolar myogenic mechanisms. G proteins are activated in both smooth (372) and skeletal muscle (349) following mechanical stimulation, and this response may occur as early as 1 min (117). In addition, a role for PLC in mechanotransduction processes has been suggested in diverse cell types including lens epithelial cells, osteoblasts, intestinal smooth muscle, and endothelial (21, 76) cells. Studies of the involvement of PLC in the arteriolar myogenic response have been limited to examining the effects of a putative inhibitor, U-73122 (173, 282), and the accumulation of the breakdown products of polyphosphoinositides. With respect to the former approach, Osol et al. (282) reported that the PLC inhibitor blocked isolated cerebral arteriolar myogenic responses, whereas Inscho et al. (173) demonstrated attenuation of pressure-induced afferent arteriolar vasoconstrictor responses. Specificity of the U-73122 inhibitor has been questioned because effects on Ca^{2+} entry, unrelated to PLC, have been reported (354). The data are supported, however, by in vivo studies of rat cremaster muscle arterioles utilizing other agents such as 2-nitro-4-carboxyphenyl-*N,N*-diphenylcarbamate and neomycin, which also inhibit PLC and are structurally unrelated to the steroid-based U-73122 compound (although neomycin also inhibits Ca^{2+} channels; Ref. 219).

Narayanan et al. (261), using pooled segments of cannulated dog renal arcuate arteries, directly measured the production of IP_3 and diacylglycerol in response to intraluminal pressure (0, 60, and 120 mmHg). Inositol trisphosphate was estimated using a specific binding assay and diacylglycerol by its conversion to labeled phosphatidic acid in the presence of diacylglycerol kinase. Both IP_3 and diacylglycerol were shown to increase with the level of intraluminal pressure and to remain elevated for the duration of the pressure increase. Because the time points studied were limited to 90 s and 15 min after application of the pressure stimulus, it is difficult to be certain that either of these mediators plays a direct role in myogenic constriction. As with a number of other potential signaling molecules, both IP_3 and diacylglycerol have been implicated in multiple signaling pathways including growth responses (309). Despite this, the data do indicate that in renal vessels intraluminal pressure can increase PLC activity, and the potential exists for either or both IP_3-mediated Ca^{2+} release or an action of diacylglycerol (for example, activation of PKC or as a source of AA) to be involved in myogenic constriction.

As with a number of the other signal transduction enzymes, however, caution must be taken in interpreting studies using broad-based inhibitors. Phospholipase C is known to exist as at least 10 isozymes characterized into three families: β, which are G protein linked; γ, which are typically activated by tyrosine phosphorylation; and δ, for which the activation process is yet to be determined (323). Furthermore, the activation mode for a given isozyme may vary among tissues (68).

Although the generation of IP_3 requires the activity of PLC, diacylglycerol can be produced from both the action of PLC on phosphatidylinositol and PLD on phosphatidylcholine. Phospholipase D-mediated production of diacylglycerol has been demonstrated to occur in response to agonists such as NE (189) and vasopressin (292). Interestingly, Narayanan et al. (261) found an excess production of diacylglycerol relative to IP_3 in pressurized renal vessels. This is suggestive of the action of an additional pathway for the generation of diacylglycerol such as that utilizing PLD. The possible involvement of PLD in myogenic signaling has not been studied further to date, although its involvement in mechanotransduction is supported by studies showing increased phosphatidylcholine hydrolysis in endothelial cells exposed to cyclical strain (82).

Given that a pressure stimulus leads to activation of PLC, two additional questions should be considered. *1*) What is the mode of activation of the plasma membrane enzyme? *2*) What are the roles of polyphosphoinositide

products (IP_3 and diacylglycerol) in the myogenic response? The latter is covered in sections IV*C1* and IV*C4*, respectively. Activation of PLC typically occurs through a trimeric G protein-coupled mechanism; however, knowledge of such a mechanism in the arteriolar myogenic response is limited. Attempts have been made to examine the effects of G protein inhibitors such as pertussis toxin and cholera toxin on myogenic responsiveness; however, such an approach is made difficult by the likelihood that multiple pathways may be simultaneously inhibited. Thus, although pertussis toxin was shown to inhibit myogenic tone in isolated cerebral arterioles (282), a similar effect may result from inhibition of the adenylate cyclase inhibitory protein, G_i, with resultant accumulation of cAMP and vasodilation rather than demonstrating a specific effect on myogenic contraction. In earlier studies, Tanaka et al. (342) reported that stretch-dependent responses of canine cerebral artery strips were unaffected by either pertussis toxin or cholera toxin, indirectly suggesting the possible involvement of G proteins other than those affecting adenylate cyclase. Although specific data are lacking, a likely candidate would be G_q, a G protein known to activate PLC. In further support of G protein involvement, the nonspecific G protein activator NaF has been shown to increase myogenic reactivity of cremaster muscle first-order arterioles (232). However, as with the inhibitor studies, it cannot be determined whether this observation reflects a specific effect on the myogenic signaling pathway or results from the fact that NaF induces contraction per se.

Although the above studies implicate a role for a G protein-PLC axis, no information exists as to how the mechanical stimulus provided by a change in intraluminal pressure is linked to G protein activation. In the case of agonist stimulation, this coupling is mediated by a classical cell surface protein receptor. In contrast, a recent study has suggested that G proteins may be directly activated by an effect of physical forces on the phospholipid bilayer component of the cell membrane (116). With the use of an artificial lipid bilayer system, it was demonstrated that physiologically relevant levels of fluid shear stress result in G protein activation. Whether such results are relevant to the effects of transmural pressure on arteriolar smooth muscle remains to be determined.

In addition to trimeric G proteins, small-molecular-weight, or monomeric, G proteins have been implicated in cellular mechanotransduction processes and modulation of smooth muscle contractile protein Ca^{2+} sensitivity. This family of some 50 related proteins includes members such as Ras, Rho, and Raf. In a similar manner to the α-subunit of the trimeric G proteins, monomeric G proteins are activated by the binding of GTP, which then enables them to exert an influence on particular intracellular effector proteins (for review, see Ref. 4). Although there are currently no data regarding a role for small-

molecular-weight G proteins in arteriolar myogenic signaling, Sadoshima and Isumo (309) have demonstrated that GTP binding of p21^{ras} occurs within 1 min of applying a stretch stimulus to neonatal cardiac myocytes cultured on a deformable substratum. Furthermore, p21^{rho} and p21^{ras} have been shown to lead to activation of mitogen-activated protein (MAP) kinases (94; see sect. IV*D3*) and also to increase smooth muscle Ca^{2+} sensitivity either directly or after agonist stimulation. Thus the potential appears to exist for such pathways being involved in arteriolar myogenic signaling.

6. Adenylate cyclase

Evidence has been presented for the involvement of the adenylate cyclase/cAMP/protein kinase A signaling pathway in mechanotransduction for several cell types (for example, Refs. 47, 360); however, there is little evidence of a primary role for adenylate cyclase in arteriolar myogenic signal transduction. Given that the generation of cAMP is typically inhibitory on myogenic tone, it would have to be argued that if adenylate cyclase was to be involved in the myogenic response, then a pressure stimulus should be associated with a withdrawal of cyclase activity. Consistent with this, Mills et al. (248), in a study of cultured coronary artery VSM cells, demonstrated that cells grown under conditions of cyclical strain showed reduced activity of adenylate cyclase. In a later study, this group extended this observation to show that chronic cyclical strain (24 h) not only decreased basal and stimulated cAMP levels, but also led to appropriate changes in adenylate cyclase-coupled G proteins (G_s and G_i) (372); thus mechanical stimulation was associated with increased G_s levels, whereas G_i activity remained unchanged. On the basis of an inverse relationship between the degree of cell stretch and adenylate cyclase activity, these authors suggested that such a signaling mechanism may be involved in the myogenic response in addition to possible chronic adaptive responses to changes in perfusion pressure.

7. Enzymes involved in the metabolism of AA

As a result of numerous studies describing roles for metabolites of AA in the regulation of vascular tone, there has been considerable interest in the possible involvement of these fatty acid derivatives in the signaling pathways underlying arteriolar myogenic reactivity. Evidence has been provided for their direct participation in the signaling process (136) and as secondary modulators (144) of myogenic tone. The possible involvement of such metabolites is strengthened by the suggestion that activation of phospholipase enzymes (see sect. IV*C5*) during a myogenic response would liberate the required AA from membrane phospholipids. An outline of the metabolic pathways involving AA is shown in Figure 9.
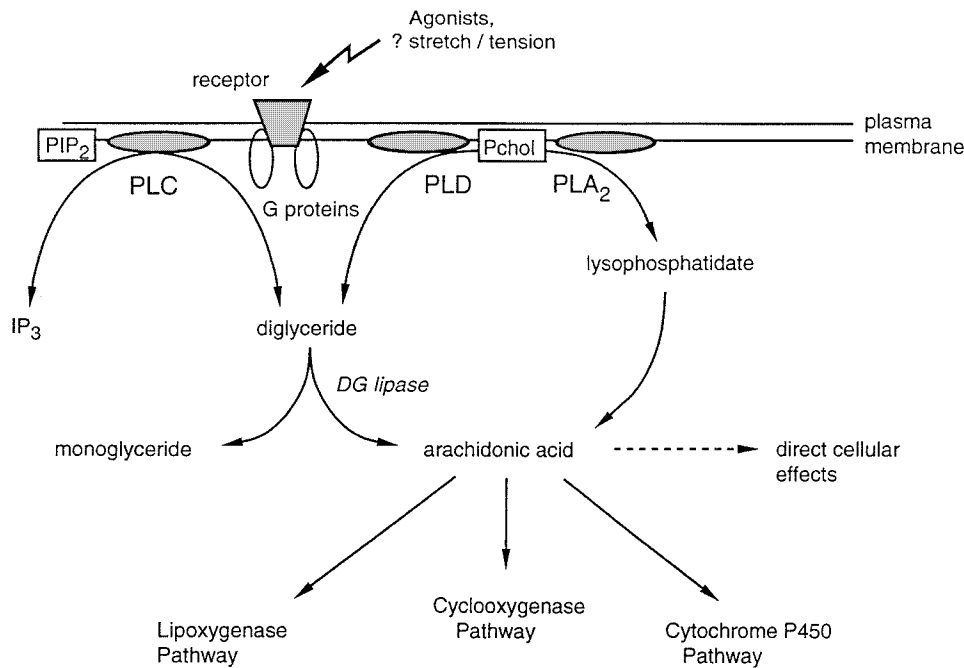
FIG. 9. Outline of metabolic pathways involving arachidonic acid (for details, see text). PLC, phospholipase C; PLD, phospholipase D; PLA_2, phospholipase A_2; IP_3, inositol 1,4,5-trisphosphate; Pchol, phosphatidylcholine.

Arachidonic acid is typically released indirectly following stimulus-induced activation of phospholipase enzymes that participate in the hydrolysis of membrane phospholipids. Arachidonic acid can then be utilized by several metabolic pathways, including those initiated by cyclooxygenase, lipoxygenase, and cytochrome *P*-450 enzymes (see Refs. 136, 137). Evidence also exists for a direct role of AA as an intracellular second messenger through mechanisms relevant to the contraction of VSM (99, 109). The cyclooxygenase pathway leads to the formation of well-characterized prostaglandin and thromboxane species. Although it is evident that such AA metabolites, generated in response to physical or chemical stimuli, can modulate basal tone, these lipid products do not appear to play a role intrinsic to the myogenic signaling mechanism. Similarly, there is no evidence for a direct role of leukotriene species, although such substances typically act as constrictor agents on arterioles.

A number of recent studies have suggested a major role for cytochrome *P*-450 enzyme metabolites, in particular 20-HETE, in the setting of myogenic tone (134, 136, 196, 398). This suggestion is based on in vitro data demonstrating that 20-HETE is an inhibitor of K_{Ca} channels, constricts arterioles at nanomolar levels, and is released from cerebral and renal arterioles in a pressure-dependent manner. Under in vivo conditions, inhibition of 20-HETE production impairs autoregulation of renal blood flow (399). Furthermore, molecular approaches have been used to show that the appropriate mRNA and protein (105) can be identified in VSM, strengthening the possibility that this factor could act on a local basis. As a result of its action on K_{Ca} channels, 20-HETE would help maintain depolarization of VSM and thereby potentiate myogenic constriction. In support of such a mechanism, Wesselman et al. (368) in studies of small mesenteric arteries have also proposed that an increase in intraluminal pressure results in inhibition of K_{Ca} channels; these authors did not, however, characterize the mediator(s) involved.

Despite the attractiveness of the *P*-450 hypothesis, several questions remain to be answered. What membrane events lead to 20-HETE production? If it is, indeed, a principal myogenic factor, then how is its production maintained after a steady-state myogenic constriction is reached? Current approaches do not allow temporal aspects of its production to be followed. It is clear, however, that 20-HETE production cannot be simply linked to overt cell length or the degree of cell stretch because, as stated earlier, the steady-state response to a given pressure increase may result in a diameter less than that before the pressure increase. A further possible complication is that endothelial cells can metabolize 20-HETE to additional vasoactive lipid species (315). Presumably, if this action of the endothelium contributed significantly, then removal of the endothelium in cannulated vessels exhibiting myogenic tone should be associated with a change in tone; this is, however, not typically observed (see Refs. 24, 241 for review). An additional question relates to how to reconcile pressure-induced production of 20-HETE and inhibition of K_{Ca} channels with studies suggesting that during steady-state myogenic tone there is activation of K_{Ca} channels and subsequent hyperpolarization; this could act as a negative-feedback mechanism to limit volt-

age-gated Ca^{2+} entry and reduce cytoplasmic Ca^{2+} (207, 265).

Recent studies have suggested that AA itself may be a second messenger with the potential to modulate VSM contraction. Arachidonate has been shown to be an inhibitor of myosin phosphatase and is released during agonist-induced contraction, thus making it a candidate as a Ca^{2+}-sensitizing factor (99, 109). Furthermore, it has been implicated in the fatty acid modulation of ion channels (see sect. IV*A4*). The direct relevance of these actions of AA to the arteriolar myogenic signaling mechanism is uncertain at present. However, if phospholipase enzymes are mechanically activated, it is reasonable to suspect that this fatty acid may directly or indirectly contribute to myogenic reactivity.

D. Cytoskeleton and Extracellular Matrix

One proposed structure for force transduction is the region of the VSM plasma membrane near the dense plaque that is in series with, and serves as an attachment site for, contractile proteins (186). As the VSM cell contracts against an external load, these attachment sites would bear the force developed by contractile elements. Although no functional data support this idea, it is interesting that both extracellular matrix and cytoskeletal proteins are concentrated at the dense plaques (193, 288), and both have been implicated in mechanotransduction (181, 382) (see sect. IV*D2*). Furthermore, dense plaque proteins are phosphorylated during smooth muscle contraction (288).

Only a few studies have addressed the issue of how mechanical forces might be transmitted though the extracellular matrix to arterioles and thereby initiate or modulate myogenic responsiveness. Measurements of the mechanical properties of the interstitium around arterioles (in situ) suggest that arterioles somehow modify the extracellular matrix in their immediate vicinity; this alters the effective wall stress of the arteriole (120) that in turn alters reactivity (110). Smooth muscle tone has also been shown to acutely modify the residual strain of arteries, possibly through connections to the extracellular matrix

(395). These observations suggest that there is a two-way interaction between the extracellular matrix and VSM force development.

1. Integrins

An attractive candidate system for transducing the physical stimulus provided by intraluminal pressure would be a mechanism whereby the extracellular matrix is coupled through the VSM plasma membrane to the cytoskeleton and contractile apparatus (355) (see Fig. 10). Integrins are a class of membrane-spanning heterodimeric proteins potentially suited to this function. Integrins are composed of α,β-heterodimers with large extracellular domains for binding matrix proteins and short cytoplasmic tails. The association of some β-integrin tails with cytoskeletal proteins such as α-actinin and paxillin (318), and with signaling proteins such as focal adhesion kinase (171), makes integrins candidates for mechanotransduction. Recent studies have demonstrated that integrins can transduce mechanical forces across the cell membrane (355) and initiate a number of signaling events, including changes in intracellular pH and $[Ca^{2+}]_i$ (325).

Most work related to integrin-mediated mechanotransduction has focused on processes related to cell growth, migration, and spreading. However, integrin-binding peptides, such as those containing the integrin-specific arginine-glycine-aspartate (peptide) (RGD) sequence, have recently been shown to inhibit myogenic tone of skeletal muscle arterioles (249). In patch-clamp experiments on myocytes isolated from those arterioles, RGD peptides were found to inhibit L-type Ca^{2+} current through interactions with the $\alpha_v\beta_3$-integrin (382). Soluble matrix proteins, such as fibronectin and vitronectin, also dilated arterioles and inhibited myocyte Ca^{2+} currents. The insoluble form of vitronectin, which binds only the $\alpha_v\beta_3$-integrin, also inhibited Ca^{2+} current, but insoluble α_5-antibody, which binds only the $\alpha_5\beta_1$-integrin, potentiated Ca^{2+} current (382). These results demonstrate that $\alpha_5\beta_1$- and $\alpha_v\beta_3$-integrins are linked to signaling pathways that bidirectionally modulate Ca^{2+} influx in VSM. They also indicate that at least part of the basal current through
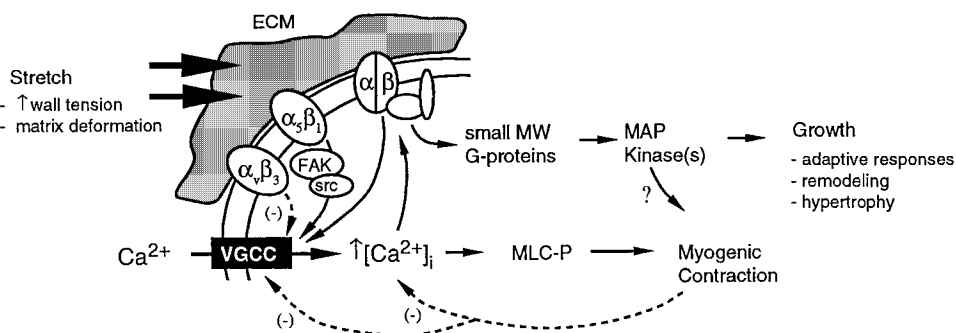

FIG. 10. Possible vascular smooth muscle mechanotransduction mechanisms involving extracellular matrix and integrins. Postulated convergence of myogenic and growth-related pathways is shown (for details, see text). ECM, extracellular matrix; MW, molecular weight; MAP, mitogen-activated protein; MLC-P, myosin light-chain phosphorylation; FAK, focal adhesion kinase; VGCC, voltage-gated Ca^{2+} channel.

L-type Ca^{2+} channels in VSM depends on existing integrin-matrix interactions, which has obvious implications for the myogenic response. Whether VGC channels or other channels in VSM are modulated by mechanical forces transmitted acutely through integrin-matrix attachments remains to be determined.

2. Cytoskeleton

Cytoskeletal stiffness is known to increase in proportion to the stress applied through integrin attachments (356), and cytoskeletal organization is known to be regulated by $[Ca^{2+}]_i$. Although most information about these processes is derived from cultured fibroblasts and epithelial cells, the relevance of this topic to VSM mechanotransduction is an emerging area of research. Adherent cells in culture form linkages between their substratum and their filamentous actin cytoskeleton at focal adhesions. A number of proteins are localized to focal adhesions, including talin, vinculin, paxillin, and α-actinin (171). The dense plaques of smooth muscle appear to be analogous to focal adhesions in cultured cells, with respect to both function and complement of associated proteins (193).

One potential role of the VSM cytoskeleton is in control of ion channel gating. In mammalian cells, the cytoskeleton has always been assumed (389) to be involved in regulation of MS ion channels because the bilayer of eukaryotic cells does not bear enough stress to directly control channel gating, except during cell lysis (307, 308). Although not definitive evidence in itself, the time- and use-dependent behavior of MS channels, including those described in smooth muscle, is consistent with the known viscoelastic properties of the cytoskeleton (307, 356). More direct evidence includes the following observations: Ca^{2+}-dependent inactivation of Ca^{2+} current in neurons is related to cytoskeletal integrity (182); the gating mode of cardiac Na^+ channels depends on an intact actin cytoskeleton (346); and disruption of endogenous actin filaments activates a K^+ current in retinal neurons (234). Although these studies are not directly related to smooth muscle, they point to the likely possibility that cytoskeletal control of channel gating is a widespread phenomenon. In the only relevant study in a smooth muscle cell line, activity of a histamine-activated, large-conductance K^+ channel was enhanced by depolymerization of F-actin using cytochalasin B (79); because the actions of these two agents were not additive but saturative, histamine (and possibly other agonists) may act through the cytoskeleton to disinhibit channel activity. It would seem inevitable that other ion channels in smooth muscle will be found to be modulated by cytoskeletal assembly and disassembly.

A few studies relevant to the cytoskeleton in intact blood vessels are worth noting. Disruption of microtubules induces shortening of cultured VSM cells and potentiates agonist-induced contraction of isolated rat pulmonary arteries (320). Microtubule disruption also potentiates phenylephrine-induced vasoconstriction in rat mesentery (223). With regard to microvessels, treatment of cannulated arterioles with microtubule-depolymerizing agents produces a time-dependent increase in arteriolar tone that parallels the disruption of the microtubule system (291). In rat aorta, neither blockade of microtubules nor actin polymerization alters the passive length-tension relationship, but both interventions reduce active force development over the entire working range of preload. In pial arteries, inhibition of actin polymerization was without effect on the initial level of myogenic tone but rendered arteries less capable of developing additional myogenic tone to elevated pressure (45). Collectively, these results suggest that both the microtubule system and the actin cytoskeleton play a role in active force development by VSM.

3. Protein tyrosine phosphorylation pathways

Phosphorylation cascades involving tyrosine phosphorylation are initiated in diverse cell types by a wide range of stimuli including growth factors, cytokines, and a variety of environmental stresses (e.g., heat, hyperosmolality, shear stress, and stretch). Increases in tyrosine phosphorylation, specifically related to mechanotransduction, have been demonstrated in cultured cardiac myocytes subjected to stretch (309) and endothelial cells under conditions of shear stress (88, 256).

Studies from DiSalvo and co-workers (67, 69) and Hollenberg (157) have suggested a possible role for tyrosine phosphorylation mechanisms in the regulation of smooth muscle contraction. In support of this suggestion, it has been shown that smooth muscle has tyrosine kinase ($pp60^{c-src}$) activity 500–700 times that of either skeletal or cardiac muscle (66). Furthermore, the tyrosine phosphatase inhibitor vanadate caused contraction that could be inhibited by the tyrosine kinase inhibitor genistein and was associated with tyrosine phosphorylation as identified by gel electrophoresis/immunoblotting.

Data concerning the specific involvement of tyrosine phosphorylation events in arteriolar myogenic signaling are currently limited and, as with MLCK, speculation as to the role of such mechanisms currently requires extrapolation from studies of large vessels. Mitogen-activated protein kinase has been implicated in thin filament contractile protein regulation through control of the phosphorylation state of the actin and myosin binding protein caldesmon (2). The implicated MAP kinases, also known as extracellular signal-regulated kinases (ERK), are serine-threonine kinases with molecular masses of 42 and 44 kDa (94). Expression of the caldesmon kinase activity appears to be dependent on both serine/threonine and

tyrosine phosphorylation of MAP kinase. The possible relevance of this mechanism to arteriolar myogenic reactivity is highlighted by several additional observations: *1*) in arterial strip preparations, MAP kinase activity increases with applied load or stretch; *2*) smooth muscle MAP kinase itself appears to be activated by phosphorylation at both threonine and tyrosine residues by a mechanism in part involving G protein kinase C (see above); and *3*) extracellular matrix-integrin binding results in the downstream activation of MAP kinases (253). Of further possible relevance to the myogenic response is the observation of Katoch and Moreland (195) that membrane depolarization in arterial smooth muscle leads to MAP kinase activation. If such a mechanism exists in arteriolar smooth muscle, it could provide a link between stretch/tension-induced membrane depolarization and activation of intracellular signaling mechanisms that modulate events such as contractile protein Ca^{2+} sensitivity or thin filament-based regulation.

In a study examining the possible role of MAP kinase-induced caldesmon phosphorylation in stretch activation of porcine carotid artery, Franklin et al. (95) have described the temporal aspects of MAP kinase activation. These authors showed that the increase in MAP kinase activity had reached its peak within 30 s of applying a load to the artery strips. Furthermore, increased MAP kinase activity paralleled or preceded the development of tension in response to KCl or the PKC activator phorbol 12,13-dibutyrate. Although this suggests a role for such kinase activity in smooth muscle contractile responses, it was also observed that a period of 60 min was required for MAP kinase activity to return to baseline after removal of the load. This would appear to indicate that if tyrosine phosphorylation plays a role in smooth muscle contraction, it is likely to do so through a supporting pathway acting in parallel to an obligatory mechanism such as MLC phosphorylation. If a primary role is played in myogenic signaling, dephosphorylation would likely need to occur rapidly as the load was removed.

Studies of isolated cremaster muscle arterioles have supported a possible role for tyrosine phosphorylation mechanisms in modulation of contractile activity. Inhibitors of tyrosine phosphorylation, genistein and tyrphostin A47, cause a concentration-dependent dilation of arterioles with spontaneous tone, and the phosphatase inhibitor vanadate causes contraction (336). The effects of the inhibitors would appear to be specific, since these agents exert their effects through different mechanisms (at the ATP binding site compared with the catalytic domain) and the inactive genistein analog diadzein is without effect. With respect to effects on $[Ca^{2+}]_i$, the inhibitors cause a decrease in $[Ca^{2+}]_i$ while the vanadate-induced contraction is Ca^{2+} dependent, being rapidly reversed by removal of extracellular Ca^{2+}. An effect of these agents on Ca^{2+} entry is supported by electrophysiological studies performed on isolated VSM cells (166, 227, 374-376, 382) and functional studies of conduit vessels (114, 344). Despite the vasodilator effects of genistein and tyrphostin A47, arterioles continued to exhibit pressure-dependent myogenic reactivity. If the inhibitors were used at sufficient concentration to cause near-maximal dilation, then myogenic reactivity was inhibited; this, however, is probably a reflection of the mechanical state of the vessel because maximal dilation with agents such as adenosine also prevents myogenic responsiveness. From the cremaster data it could be argued that, although tyrosine phosphorylation pathways are present in arteriolar smooth muscle and may modulate tone through an effect on Ca^{2+} availability, they are not intrinsic to the myogenic mechanism.

As with PKC, even if tyrosine kinase-mediated mechanisms are involved in myogenic signaling, little information is available as to the actual proteins phosphorylated by these enzymes. Whether such substrates might be involved in modulation of Ca^{2+} availability or might directly affect the contractile process needs to be determined in future studies. Stimulation of airway smooth muscle by ACh results in serine-threonine phosphorylation of talin and tyrosine phosphorylation of paxillin; the time course of phosphorylation of these dense-plaque proteins exactly parallels the time course of force development (288). The temporal pattern of phosphorylation is particularly significant, since many tyrosine phosphorylation events have been associated with growth pathways (385). By analogy to the receptor-mediated agonist angiotensin II, which stimulates both contraction and tissue growth, it is conceivable that the stimulation of tyrosine phosphorylation by an increase in arteriolar intraluminal pressure may reflect a process underlying longer term adaptive responses within the vessel wall, rather than being integral to the acute myogenic contractile process. A few of the possible tyrosine phosphorylation-mediated effects on myogenic and cell growth responses are illustrated in Figure 10. Further studies are required to determine whether such signaling mechanisms function independently or if significant interaction occurs. Interestingly, in recent studies, Allen and co-workers (6, 7) have shown in cannulated mesenteric arterioles that an increase in intraluminal pressure leads to protooncogene expression (taken as an indicator of a growth response) in vessels behaving passively, whereas gene expression was attenuated in vessels demonstrating myogenic constriction. Conceivably, this could be explained by mechanical effects as the vessel contracts (e.g., decreasing wall tension) or by differences in the concentrations of intracellular mediators produced during steady-state myogenic contraction as compared with that of the passive vessel.

V. FUTURE DIRECTIONS FOR RESEARCH ON MYOGENIC MECHANISMS

Over the last 20 years, significant advances have been made in our understanding of the mechanisms underlying the vascular myogenic response. Despite these advances, many questions remain to be answered, and many controversies need to be resolved. Certainly, differences in species, tissue, vessel size, and method of study contribute to the variability in many of the results described above. However, it would seem unlikely that a phenomenon as basic as the myogenic response, present in almost every type of arterial vessel (to some degree), would utilize different signaling pathways in different types of vessels.

With respect to mechanical properties of smooth muscle, is stretch activation of isometric preparations an equivalent mechanical stimulus to pressure-induced constriction of cannulated vessels? Does shortening deactivation in an isometric preparation reflect the same underlying mechanism as myogenic dilation of a pressurized arteriole? Is the myogenic behavior of arterioles simply an extension of the same mechanical response to length changes seen in striated muscle? If so, this might diminish the importance of studies related to specific membrane-bound receptors and contractile proteins. Comparative mechanical and mechanistic studies using both isometric and isobaric techniques may allow us to distinguish between the numerous terms used to describe myogenic tone and myogenic responsiveness. The wall tension hypothesis needs to be tested experimentally. Do arterioles have specific biochemical or electrophysiological mechanisms for enhancing their myogenic responsiveness, or is the greater magnitude of pressure-induced constriction and dilation observed in small arterioles related to the fact that wall tension, as dictated by the law of Laplace, is more easily overcome in vessels of that size?

With respect to electromechanical coupling, is depolarization sufficient to account for myogenic behavior? The roles of MS cation channels and Cl^- channels need to be clarified. This will require the development and careful testing of specific pharmacological antagonists for the respective channels, along with parallel or simultaneous measurements of electrophysiological and mechanical responses of arterioles. The physiological relevance of the various methods used to mechanically stimulate single VSM cells needs to be evaluated. Are these stimuli more relevant to volume control than to myogenic behavior? In this regard, voltage-clamp experiments of single VSM cells in intact, pressurized arterioles would be highly desirable, but space-clamp and movement-related problems must first be overcome. The role of K^+ channels needs further clarification. What is the function of the stretch-activated K^+ channels identified using single-channel techniques? Is 20-HETE inhibition of K_{Ca} current a mechanism common to most arterial vessels? Is 20-HETE involved in initiating a myogenic contraction or just in sustaining myogenic tone? Are other metabolites of AA of equal or greater importance in producing or counteracting myogenic tone?

With respect to second messenger pathways involved in arteriolar myogenic signaling, future studies must decrease their reliance on the use of small-molecular-weight inhibitors lacking sufficient specificity. Studies of contractile filament calcium sensitivity are also limited by the lack of specific inhibitors. For example, what fraction of the inhibition of myogenic tone by PKC antagonists can be explained by effects on ion channels? Methods of delivery of peptide-based inhibitors must be developed for arterioles with intact electromechanical coupling mechanisms. Also, significant differences between the biochemistry of conduit vessels and resistance vessels point out a critical need to enhance the sensitivity of biochemical and molecular biological methods for use with extremely small samples. This will enable quantitative analysis of proteins and specific protein isozymes to be made in arterioles exhibiting substantial myogenic behavior.

Studies on mechanotransduction through the extracellular-matrix-integrin-cytoskeleton axis will likely be an important area of research over the next decade. Important questions to be addressed with regard to myogenic responses include the following: Are mechanical forces associated with arteriole pressurization transmitted specifically through integrins? What is the role of the VSM cytoskeleton? Are mechanical effects on ion channels and membrane-bound enzymes dependent on the cytoskeleton? Does tyrosine phosphorylation play a central role in determining myogenic responsiveness? If so, what specific proteins are phosphorylated? Is cultured VSM (currently required to obtain sufficient quantities of protein for molecular assays) an adequate model of intact VSM?

Finally, it will be necessary to determine which signaling mechanisms in VSM are fundamental to myogenic contraction and which represent parallel modulatory pathways. What is the relative importance of electromechanical coupling versus changes in contractile protein sensitivity to Ca^{2+}? What are the consequences of having to preactivate vessels with agonists to induce myogenic responses or tone? Which pathways are more important in determining acute responses to pressure as compared with longer term, adaptive growth responses? Answers to these questions will provide important information necessary to develop therapeutic agents that specifically enhance or inhibit vascular myogenic tone.

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