Transduction Mechanisms Involved in the Regulation of Myogenic Activity

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Abstract Vascular smooth muscle has the ability to exist in a state of maintained partial constriction, either spontaneously or in response to changes in intravascular pressure, commonly known as myogenic behavior. This behavior presents itself in several characteristic forms, including (1) myogenic tone (often referred to as basal, intrinsic, or spontaneous tone), the sustained contractile state intrinsic to vascular smooth muscle exhibited in the absence of neural or humoral influence; (2) the myogenic response, or the ability of vascular smooth muscle to constrict after an increase in intravascular pressure and, conversely, to dilate on lowering of intravascular pressure; and (3) vasomotion, the spontaneous, rhythmic oscillations in lumen diameter (see Johansson for review). Although myogenic behavior has been reported in conduit arteries and the facial vein, myogenic tone and myogenic responses are more prevalent in the resistance vasculature, in particular, within the microcirculation. In fact, detailed studies have shown that within a vascular network, relative myogenic responsiveness increases with decreasing arteriolar size.

Use of the term myogenic itself implies that mechanical stimuli act directly on vascular smooth muscle cells to elicit contraction. However, more recent evidence has shown that the vascular endothelium can modulate force production by vascular smooth muscle by virtue of its ability to release both relaxing and contracting factors in response to chemical and mechanical stimuli. Thus, endothelium-derived vasoactive agents may play a role in regulating myogenic behavior and in some cases may be responsible for pressure-dependent changes in vasoconstrictor activity previously ascribed to myogenic phenomenon. On the other hand, numerous studies have demonstrated that an intact endothelium is not the basis for normal myogenic responsiveness (see Meininger and Davis for review). This has been shown in several arterial preparations, including rat cerebral arteries, rabbit ear arteries, rat gracilis arterioles, rat cremaster arterioles, rat mesenteric arterioles, and porcine coronary arterioles.

This review will provide an overview of the cellular mechanisms currently believed to play a role in determining myogenic activity in vascular smooth muscle. The cell signaling pathways described are thought to represent some of the important steps in transducing mechanical stimuli into the myogenic behavior characteristic of vascular smooth muscle. The elucidation of these pathways will not only provide us with an understanding of an important vasomotor phenomenon but also help us understand the potential for myogenic activity to contribute to changes in vascular resistance that occur in arterial hypertension.

Mechanisms for the Initiation and Maintenance of Myogenic Tone

Modulation of Membrane Electrical Properties by Stretch

Stretch as a Mechanical Stimulus

The principal hemodynamic force to which vascular smooth muscle is exposed is a persistent distention caused by the transmural pressure gradient. With regard to myogenic activity, the pressure-induced distention or stretch is believed to be the primary stimulus responsible for the initiation and maintenance of myogenic activity. As with skeletal and cardiac muscle, smooth muscle is an electrically excitable tissue in that changes in membrane potential directly affect its contractile state. The first indication that changes in smooth muscle length could alter its electrical and mechanical activity came from the pioneering work of Bulbring and colleagues. Stretching strips of smooth muscle isolated from the guinea pig Taenia coli produced progressive depolarization of the cell membrane and tension.
development compared with unstretched preparations, leading these investigators to conclude that membrane potential correlated inversely with cell length and tension. As in *T. coli*, tracheal smooth muscle cells progressively depolarized with step increases in cell length. Because membrane potential returned toward control values despite the maintained increases in cell length, Coburn reasoned that depolarization occurred in response to the stretch itself rather than to a maintained increase in cell length. Because stretch-induced depolarization was not influenced by either atropine or tetrodotoxin at concentrations that completely inhibited field-stimulated contractions, the author concluded that this represented a myogenic rather than a neurogenic mechanism.

Like visceral smooth muscle, smooth muscle cells of the portal vein exhibit slow depolarization leading to a burst of spike potentials, which in turn correspond to rhythmic contractions. Increasing rates of stretch of rat portal vein strips likewise elicited graded increases in both electrical (frequency of spike discharge) and mechanical (isometric force) activity. Responses were unaffected by α- and β-adrenergic blockade, again ruling out the possibility of a neurogenic mechanism.

Harder and colleagues and Monos et al used a model in which isolated arterial segments were cannulated on either end of a perfusion system, thereby allowing them to measure simultaneously membrane electrical activity and changes in lumen diameter after elevations in transmural pressure. In cat middle cerebra and canine renal (interlobular) arteries, increased transmural pressure was accompanied by membrane depolarization and consequent vasoconstriction. Normalized to starting conditions at 40 mm Hg pressure, a 1.9-mV depolarization and 1% decrease in lumen diameter with each step increase in transmural pressure were noted by Harder. The slope of this relation between transmural pressure and membrane potential depended on the extracellular Ca²⁺ concentration, suggesting that an elevated Ca²⁺ conductance was responsible for myogenic activation. In addition, membrane depolarization was associated with an increase in the frequency of Ca²⁺-dependent spike potentials. As in visceral smooth muscle, membrane depolarization, vasoconstriction, and spontaneous electrical activity were independent of α-adrenergic mechanisms. Collectively, these data suggest that pressure-induced increases in Ca²⁺ entry and membrane depolarization were linked to activation of vascular smooth muscle.

**Transduction of Stretch Stimulus: Stretch-Activated Channels**

Recently, investigators have shown that a class of ion channels sensitive to membrane deformation, or mechanosensitive channels, initiates mechanoelectric transduction (reviewed in Morris). Among the subtypes included in this category of channels are those that are sensitive to membrane deformation imposed by cell stretching, referred to as stretch-activated channels (SAC). First identified in cultured embryonic chick skeletal muscle, SAC have been characterized in a variety of cell types, ranging from prokaryotes to eukaryotes. Within the latter group, SAC have been characterized in fungi and plants and on up through members of the animal kingdom. In association with their widespread distribution, SAC have been implicated in such diverse functions as (1) regulation of cell volume and osmoregulation, (2) transduction of auditory stimuli, (3) modulation of neurite elongation, (4) remodeling of bone tissue by activation of osteoblasts in response to mechanical stresses, and (5) modulation of smooth muscle activation and contractility. The ability of SAC to modulate the contractile state of vascular smooth muscle has implicated a role for them in the myogenic mechanism.

**Stretch-Activated Channels in Smooth Muscle**

With regard to SAC and myogenic phenomena, patch-clamp techniques have been used to record single-channel currents in enzymatically dispersed smooth muscle cells. By application of additional suction to the patch pipette after gigaseal formation, membrane tension could be altered, the extent of which could be calculated using Laplace's law. Using this technique, Kirber et al characterized a cation nonspecific SAC in visceral (toad stomach) smooth muscle having unitary conductances for Na⁺, K⁺, and Ca²⁺ of 58, 66, and 18.5 pS, respectively. Because of the high density in the cell membrane and cation selectivity, these authors postulated that this channel may play a role in mechanoelectric transduction that initiates stretch-induced contraction of smooth muscle.

Like vascular (porcine coronary artery) smooth muscle Davis et al identified a SAC having a similar relative order of selectivity for Na⁺ (23 pS), K⁺ (36 pS), and Ca²⁺ (11 pS). In normal physiological saline solution stretch-activated whole-cell current reversed at approximately −15 mV, indicative of a non-specific cation channel. In addition, Davis et al recorded changes in membrane potential as a function of cell stretching using the whole-cell configuration. To do so, they took advantage of the mechanical stability imparted by gigaseal formation of the patch pipettes to hold one portion of the cell in place, while they positioned a second pipette at the opposite end of the cell to impose the cell stretch. They used a third microelectrode placed at the stationary end to record membrane potential or whole-cell current while the cells were stretched to varying degrees above their slack length. An important aspect of these observations was that the relation between membrane potential and change in cell length was similar to that previously found by Harder for membrane potential and transmural pressure; that is, membrane depolarization accompanied increases in cell length.

Typically, membrane depolarization elicits the influx of Ca²⁺ through ion channels sensitive to changes in membrane potential, or voltage-dependent Ca²⁺ channels (VDCC). The ensuing rise in intracellular Ca²⁺ concentration ([Ca²⁺]) precipitates the enzymatic cascade leading to muscle shortening or force production. The results of several studies, however, indicate that stretch-activated Ca²⁺ influx occurs at least in part through a channel distinct from VDCC. The earliest studies were performed on wire-mounted arteries that, after equilibration, were stretched under zero extracellular Ca²⁺ conditions. After the initial increase in passive tension, total tension declined as a result of stress relaxation and reached a steady state above initial
levels. At this point, Ca\(^{2+}\) was added and the development of force recorded; this force was referred to as myogenic tone. In rabbit ear resistance artery and basilar artery, concentrations of VDCC inhibitors that fully blocked contraction to KCl depolarization were ineffectual in altering the development of myogenic tone. Likewise, Ca\(^{2+}\) uptake in response to stretch was unaffected in the presence of the 1,4-dihydropyridine PN 200-110 in the rabbit facial vein, a venous preparation that paradoxically develops intrinsic tone under the appropriate conditions; conversely, Ca\(^{2+}\) influx during KCl depolarization was significantly attenuated under similar conditions. More recently, Davis et al.\(^{14}\) using the fluorescent dye fura 2 to monitor [Ca\(^{2+}\)], in single smooth muscle cells from porcine coronary artery, have shown that stretch induces an increase in [Ca\(^{2+}\)]. This response was only partially attenuated by 5 \(\mu\)mol/L nifedipine, a concentration that in a separate series of patch-clamp studies fully blocked the inward Ca\(^{2+}\) current through VDCC.

Similar results have been reported in skeletal muscle microvessels in vivo. For these studies, anesthetized rats were placed in a Plexiglas box, and the cremaster muscle was surgically prepared such that it was exteriorized into a bathing chamber to allow direct visualization of the microcirculation. A compressed air inlet port was attached to the box to permit box pressure to be rapidly changed. Altering the pressure within the box produced an equivalent change in intravascular pressure that was transmitted to the microcirculation. Arterioles responded to pressure changes with a myogenic response that was recorded as changes in lumen diameter. Using this model, M.A. Hill and G.A. Meininger demonstrated (unpublished observation, 1993) that VDCC blockade attenuated but did not abolish the pressure that was transmitted to the microcirculation. It has previously been noted that there is a pronounced myogenic response gradient in the arterial tree, with myogenic phenomena becoming much more prominent in the smaller arterioles. Within the microcirculation, it is characteristically observed that (1) there is more myogenic tone, (2) myogenic responses are larger in magnitude, and (3) myogenic responses occur with a shorter time constant (i.e., more rapidly). The basis for these differences has not been elucidated, but it would be of interest to determine whether they are founded in unique electrophysiological properties related to SAC and VDCC.

In this regard, it is interesting to note that in those studies previously mentioned in which basal tone is reversed by VDCC inhibitors, the smallest average lumen diameter measured under fully relaxed conditions was 142 \(\mu\)m.\(^{55}\) It is therefore possible that myogenic tone in these proximal resistance arteries exhibits a greater dependence on Ca\(^{2+}\) entry through VDCC than that in the microcirculation. This conclusion is supported in part by the finding of Gustafsson et al.\(^{62}\) that vasodilator responses to VDCC inhibitors were more prominent in proximal resistance arteries compared with microvascular arterioles (lumen diameter <25 \(\mu\)m).

**Voltage-Dependent Calcium Channels**

Evidence that the influx of extracellular Ca\(^{2+}\) plays an obligatory role in the maintenance of intrinsic vascular tone has been demonstrated in numerous studies. In fact, estimates of myogenic tone at a particular distending pressure (cannulated arterial segments under pressurized conditions) or level of stretch (wire-mounted preparations) are typically made by comparing lumen diameter or force, respectively, in the absence versus presence of external Ca\(^{2+}\). It is unlikely, however, that Ca\(^{2+}\) influx through SAC is responsible for the maintenance of arterial tone because of the relatively low channel density and small Ca\(^{2+}\) conductance in vascular smooth muscle.\(^{47}\) The evidence for the existence of SAC in vascular smooth muscle and the partial independence of the development of myogenic tone on VDCC has led to the proposal that the influx of extracellular Ca\(^{2+}\) through SAC initiates mechanoelectric transduction in response to changing cell length. Maintenance of myogenic tone, however, appears to depend on Ca\(^{2+}\) entry through VDCC. A model proposed by Meininger and Davis\(^{44}\) emulates that activation by stretch permits the entry of extracellular Ca\(^{2+}\) through SAC. As these channels are nonspecific cation channels, inward current is also carried by Na\(^{+}\), and it is this Na\(^{+}\) current that elicits further membrane depolarization sufficient to open VDCC.

In vascular smooth muscle Ca\(^{2+}\) current is carried primarily through VDCC, which inactivate relatively slowly and incompletely during prolonged depolarization and are sensitive to inhibition by dihydropyridines, the so-called L-type channels (reviewed in Nelson et al.\(^{51}\)). Several studies have shown that myogenic tone is sensitive to VDCC blockade. In the autoperfused cat hind limb preparation, intra-arterial infusion of the dihydropyridine felodipine produced a dose-dependent decrease in basal vascular resistance and perfusion pressure.\(^{52,53}\) Likewise, in isolated, pressurized cerebral\(^{23,54}\) and skeletal muscle\(^{55}\) resistance arteries, myogenic tone is abolished by VDCC inhibitors. Conversely, in the intact, exteriorized rat cremaster muscle, topical administration of nifedipine and methoxyverapamil to 3A arterioles (passive lumen diameter \(\leq 25 \mu\)m) produced only a small vasodilation compared with the maximal vasodilator response yielded with adenosine (M.A. Hill, G.A.M., unpublished observations, 1993).

These differences may reflect heterogeneity in regulatory mechanisms governing myogenic tone in proximal resistance arteries versus those present in the small arterioles of the microcirculation. It has previously been noted that there is a pronounced myogenic response gradient in the arterial tree, with myogenic phenomena becoming much more prominent in the smaller arterioles. Within the microcirculation, it is characteristically observed that (1) there is more myogenic tone, (2) myogenic responses are larger in magnitude, and (3) myogenic responses occur with a shorter time constant (i.e., more rapidly). The basis for these differences has not been elucidated, but it would be of interest to determine whether they are founded in unique electrophysiological properties related to SAC and VDCC.

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**Calcium-Activated Potassium Channels**

It is conceivable that as inward cationic currents through SAC and consequently VDCC depolarize the cell membrane, the decrease in membrane potential could progressively increase the open probability of VDCC and thereby cause further depolarization according to a positive feedback mechanism. Bearing in mind the relation between membrane potential and arterial lumen diameter,\(^{50,53}\) depolarization produced by an increase in transmural pressure could, in the absence of any feedback control, potentially elicit complete occlusion of the arterial lumen. It is reasonable to assume, therefore, that there exists a negative feedback pathway, or vasodilating mechanism, that is activated by
elated transmural pressure, membrane depolarization, or increased [Ca\(^{2+}\)]; to offset the depolarizing influence of SAC and VDCC.

The large-conductance Ca\(^{2+}\)-activated K\(^+\) channel (K\(_{Ca}\)) appears to have the characteristics necessary to participate in this capacity. Patch-clamp techniques have shown that this activity is dependent on both voltage and [Ca\(^{2+}\)], in visceral\(^{56,57}\) and vascular\(^{58,59}\) smooth muscle. Thus, the activation of an outward K\(^+\) current through K\(_{Ca}\) could provide a hyperpolarizing mechanism to oppose both membrane depolarization and increased [Ca\(^{2+}\)]. Recently, Brayden and Nelson\(^{44}\) have provided compelling evidence to suggest that K\(_{Ca}\) is involved in this negative feedback pathway in vascular smooth muscle. Charybdotoxin, a selective inhibitor of K\(_{Ca}\), produced dose-dependent constriction of pressurized (75 mm Hg) rabbit cerebral arteries, which was accompanied by membrane depolarization of the vascular smooth muscle. In the presence of nimodipine (0.1 \(\mu\)mol/L) or at a distending pressure of 10 mm Hg, conditions that eliminate or greatly diminish intrinsic tone, charybdotoxin had negligible effects on lumen diameter and membrane potential. Together, these observations were interpreted to demonstrate a link between Ca\(^{2+}\) entry through VDCC and subsequent K\(_{Ca}\) activation.

**Involvement of Second Messengers in the Maintenance of Myogenic Tone**

In vascular smooth muscle, agonist stimulation triggers the breakdown of membrane phospholipids to yield second messengers that elicit a rise in [Ca\(^{2+}\)]; and consequent activation of Ca\(^{2+}\)-calmodulin–dependent myosin light chain (MLC) kinase. Phosphorylation of MLC permits the activation of myosin ATPase by actin, thereby allowing crossbridge cycling, and therefore force production or muscle shortening, to occur (reviewed in Kamm and Stull\(^{60}\) and Stull et al\(^{61}\)). The finding that both [Ca\(^{2+}\)] and the extent of MLC phosphorylation increase transiently during force production yet decline to suprabasal levels during force maintenance\(^{62-65}\) has led investigators to speculate on the existence of alternate regulatory mechanisms responsible for the tonic portion of smooth muscle contraction.

It is reasonable to suggest that the maintained partial contraction of vascular smooth muscle characteristic of myogenic tone may be functionally equivalent to the tonic phase of force production observed with agonists. That some agonists, notably \(\alpha\)-adrenergic agonists, increase vascular tone and simultaneously enhance the myogenic response\(^{66-68}\) has strengthened the perception that common signaling pathways may be shared. To date, however, the extent to which MLC phosphorylation or alternate regulatory mechanisms govern intrinsic arterial tone remains unknown. To this end, several laboratories have recently begun to focus on the influence that second messengers may exert on the development and maintenance of myogenic tone. Several lines of investigation are summarized below.

**Guanine Nucleotide Regulator Proteins**

Guanine nucleotide regulatory proteins, or G proteins, are widely recognized as essential to transmembrane signaling pathways by which agonists elicit the production of intracellular second messengers (for reviews, see Birnbaumer and Brown\(^{69}\) and Gilman\(^{70}\)). Whether mechanochanical transduction involves a G protein intermediary has perhaps been investigated the least. Incubation of pressurized cerebral arteries with pertussis toxin produced a 75% reversal of myogenic tone.\(^{71}\) It is known that pertussis toxin catalyzes the ADP ribosylation of the Go subunit of certain Go classes of G proteins, thereby inhibiting their activation, whereas the Ga subunit of the G\(_i\) class is pertussis toxin resistant.\(^{72}\) Normally, stimulation of Go inhibits adenylate cyclase; removal of inhibition by pertussis toxin would therefore permit the unopposed basal activity of adenylate cyclase and consequent accumulation of cyclic AMP (cAMP), an effect similar to that of papavarine, which is a phosphodiesterase inhibitor. Although it is disputed whether cAMP causes relaxation via a decrease in [Ca\(^{2+}\)]\(^{73}\) or by increasing the Ca\(^{2+}\) requirement for force maintenance,\(^{74}\) pertussis toxin nevertheless produced a vasodilator response, presumably through disinhibiting adenylate cyclase.\(^{74}\) These data therefore suggest that the maintenance of myogenic tone is mediated at least in part by the tonic inhibition of adenylate cyclase.

Using a model in which force production was recorded after a quick stretch of helical strips of canine cerebral arteries, Nakayama and Tanaka\(^{75}\) also tested responses in the presence of G protein inhibitors. These investigators had found that neither cholerotoxin, which catalyzes the ADP ribosylation of the Go subunit of the G\(_i\) class of G proteins, nor pertussis toxin had any effect on stretch-induced contraction. These data suggest that another type of G protein, possibly G\(_i\), may be involved in the transduction of stretch into mechanical shortening. It could be argued, however, that the apparent discrepant results could reflect distinct mechanisms governing the maintenance of arterial tone and the vasoconstrictor response observed after a step change in pressure (myogenic response).

Conversely, stimulation of G proteins with NaF, a nonspecific G protein activator, augments basal tone of isolated cerebral arteries pressurized to 75 mm Hg\(^{76}\) and of exteriorized rat cremaster 1A arterioles.\(^{76}\) Unfortunately, these results do not distinguish between activation of a G protein associated with the transduction scheme of Ca\(^{2+}\) mobilizing agonists (G\(_q\)) versus that G protein which inhibits adenylate cyclase (G\(_i\)). As an additional note of caution, however, the finding that G protein activation can enhance basal tone does not in and of itself support any role for G proteins in the maintenance of myogenic tone. As G proteins are involved in the transduction pathway for vasoconstrictor agonists, G protein activation is functionally equivalent to agonist stimulation, the only exception being that NaF bypasses the receptor.

**Inositol 1,4,5-Trisphosphate and Calcium Release**

Agonist stimulation of vascular smooth muscle involves the hydrolysis of the plasmalemmal phospholipid phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) by the membrane-bound enzyme phospholipase C (PLC) to yield the second messengers inositol 1,4,5-trisphosphate (IP\(_3\)) and diacylglycerol (DG). IP\(_3\), released into the cytoplasm where it causes the release of Ca\(^{2+}\) from intracellular stores and initiates phasic contraction. Diacylglycerol, on the other hand, remains in the plasma
membrane and leads to activation of protein kinase C (PKC) (reviewed in Berridge77).

Several studies have previously demonstrated that, like agonists, mechanical activation stimulates the production of inositol phosphates and subsequent release of Ca2+ from intracellular stores. Using flexible culture plates, stretch cultured pulmonary vascular smooth muscle cells, Kulik et al80 have shown that a single stretch produces a 34% and 58% increase in IP3 and inositol 1,3,4,5-tetrakisphosphate (IP4) levels, respectively; however, values had declined toward baseline during the maintained increase in cell length. Pressure likewise appears to be a sufficient stimulus to generate IP3 in isolated renal arterial segments.79 Consistent with these findings, rapid stretch of smooth muscle cells isolated from porcine coronary artery elicited a transient elevation in [Ca2+]i, under Ca2+-free bath conditions, which is indicative of Ca2+ release from intracellular stores.80

That mechanical stimulation can result in the production of IP3 stimulates the acidolytic PLC. Only recently, however, has the involvement of PLC in the maintenance of arterial tone been directly shown. In rat posterior cerebral arteries, vessels that developed 35% myogenic tone at a distending pressure of 75 mm Hg, U-73122, a PLC inhibitor, produced dose-dependent vasodilation.71 The authors noted that the ability of U-73122 to abolish receptor-dependent (serotonin) but not receptor-independent (KCl depolarization) vasoconstriction attested to its specificity for the receptor-mediated transduction pathway. Inhibition of PLC by 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC) similarly abolished force production after quick stretch of canine cerebral arteries.73

Recent advances in the study of cellular transduction pathways have shown that the PLC-β isoform is activated by members of the Gαq class of G proteins.80,81 To determine whether the tonic PLC activity seen in pressurized cerebral arteries is coupled to G protein activation, Oso et al71 tested the effects of PLC inhibition on arterial segments preconstricted by G protein activation with NaF. In these experiments vessels were pressurized to 30 mm Hg, at which pressure no spontaneous tone developed, thereby preventing any complications due to synergistic effects caused by pressure and agonist stimulation.82 However, U-73122 concentrations that were sufficient to abolish myogenic tone of arteries pressurized to 75 mm Hg had no effect on the NaF constrictor response, suggesting that the potentiating effect of G protein activation was independent of PLC. Conversely, PLC inhibition with neomycin and NCDC shifted NaF constrictor curves of exteriorized rat cremaster 1A arterioles significantly to the right; furthermore, maximal constriction was significantly reduced in the presence of NCDC, suggesting that the effects of G protein activation are mediated through PLC.76 It is possible, however, that these differences reflect the activation of different G protein–mediated transduction pathways, although G proteins in proximal resistance arteries and microvascular arterioles have yet to be characterized either qualitatively or quantitatively.

Protein Kinase C

Although stretch or pressure may elicit the release of Ca2+ from intracellular stores and thereby cause a transient elevation in [Ca2+]i, it is unlikely that this mechanism in and of itself is responsible for the maintenance of myogenic tone. For this to occur, stretch or pressure that initiates the development of myogenic tone must also be accompanied by sustained increases in [Ca2+], supported by enhanced Ca2+ entry or by eminence of events that sensitize contractile proteins such that arterial tone can be maintained at a lower [Ca2+], than that required to initiate the development of myogenic tone. PKC has been proposed to play a role in the Ca2+-force relation such that force can be maintained at lower [Ca2+], (see below for references). For this reason, several laboratories have explored a possible role for PKC in myogenic phenomena. As previously stated, diacylglycerol is the other second messenger produced by PLC-mediated hydrolysis of PIP2. Because Ca2+-mobilizing agonists such as carbachol82 and angiotensin II83 induce a sustained increase in diacylglycerol in smooth muscle cells, it had been postulated that PKC plays a regulatory role in the maintenance of isometric force production. This contention is supported by the fact that activation of PKC by phorbol esters elicits a slowly developing yet sustained contraction of vascular smooth muscle.84-90 Although the mechanism by which PKC activation produces force in smooth muscle is highly disputed, perhaps both species and tissue specific, it has been demonstrated that phorbol esters (1) sensitize the contractile apparatus to [Ca2+]i, such that lower [Ca2+]i, is required for force maintenance85,91-93 and (2) modulate Ca2+ channel activity.85,94,95

The recent finding that suggests PLC is tonically active in pressurized arteries that exhibit myogenic tone71 implies that diacylglycerol would be generated continuously under basal conditions. This further suggests that PKC participates in the maintenance of intrinsic arterial tone. Previously, pharmacologic studies from several laboratories have provided evidence supporting such a role for PKC. In stretched ring segments from rabbit facial vein and basilar artery96 incubated in Ca2+-free saline solution, treatment with staurosporine, a PKC inhibitor, attenuated the development of myogenic tone after the readmission of Ca2+ to the media. Pretreatment of ferret aortic strips, a model that exhibits temperature-dependent intrinsic tone, with staurosporine was reported to cause a significant decrease in the level of tone.9 In addition, staurosporine has been found to produce a dose-dependent inhibition of basal arterial tone in isolated, pressurized rat cerebral arteries.97 Conversely, Hill et al98 using the exteriorized rat cremaster model, found that topical application of the PKC inhibitors H-7 [1-(5-isouquinolinylsulfonyl)-2-methylylperazine hydrochloride; up to 10 μmol/L] and staurosporine (up to 0.1 μmol/L) onto 3A arterioles had no effect on basal lumen diameter; myogenic responses to step changes in intravascular pressure, on the other hand, were significantly attenuated.

Two possibilities exist that may in part explain these discrepancies. First, these conflicting results may be representative of differences inherent to in vivo versus in vitro models. In the exteriorized cremaster model for the study of arteriolar reactivity in vivo, 1A and 2A arterioles possess little myogenic tone (≤10%) and do not exhibit a myogenic response to step increases in pressure67,68,99; however, isolated 1A cremaster arteri-
oles, which were cannulated in vitro and pressurized, developed significant myogenic tone (30% to 40%) and actively constricted to increases in transmural pressure. Similar to the variability in the participation of PKC in governing myogenic tone may reflect a heterogeneity in responses along the vascular tree, as was previously discussed for the dependence of Ca\(^{2+}\) influx through VDCC. In other words, PKC appears to mediate basal tone in larger arteries but not in the microcirculation.

Interpretation of the results from those studies using PKC inhibitors, however, is complicated by their relative nonspecificity. Both staurosporine and H-7 inhibit ATP binding to the catalytic domain of PKC; however, a significant portion of the catalytic domain of PKC shows striking homology with ATP binding sites of other serine- and threonine-specific kinases, such as MLC kinase. With regard to the studies previously mentioned, concentrations of staurosporine (10 nmol/L) that completely abolished basal tone of pressurized cerebral arteries had been shown to attenuate responses to KCl depolarization in rabbit aortic rings, which presumably stimulates force production via a pathway independent of PKC. In this latter study, half-maximal and near-maximal inhibition of force production in response to depolarization was produced by 50 and 100 nmol/L staurosporine, respectively. These staurosporine concentrations severely reduced the extent of myogenic tone developed in the models used by Laher and colleagues. In these studies, therefore, the possibility that these PKC inhibitors exert direct effects on MLC kinase cannot entirely be ruled out.

**Myosin Light Chain Kinase**

Force production or cell shortening of smooth muscle is ultimately accomplished by the activation of Ca\(^{2+}\)-calmodulin–dependent MLC kinase, which in turn catalyzes the phosphorylation of the 20-kd regulatory MLC. It therefore stands to reason that stretch or pressure should lead to stimulation of MLC kinase activity during the development of myogenic tone. It has been shown that application of a quick stretch to strips of porcine carotid artery increased MLC phosphorylation, the extent of which correlated with the degree of stretch. It is curious that MLC phosphorylation levels were not sustained after tissue stretch. Despite the fact that the MLC phosphorylation level declined toward baseline within the first 10 minutes after stretch, force was maintained. This finding bears a striking resemblance to the temporal phosphorylation pattern during agonist stimulation and hence is suggestive of a "latch" state. In ferret aorta the level of MLC phosphorylation remained invariant between 21°C and 37°C, even though the extent of developed tone increased with elevated temperature; yet MLC phosphorylation was significantly depressed at 0°C, despite no change in [Ca\(^{2+}\)]. The authors reasoned that this reflects a constitutively active, Ca\(^{2+}\)-independent form of MLC kinase, as has been previously described by Ikebe et al.

Force production after either a quick stretch or stretch-release protocol, presumably caused by the activation of MLC kinase during the stretch portion, was inhibited by antagonists to calmodulin and to MLC kinase or by long-term incubation in Ca\(^{2+}\)-free buffer. Short-term treatment with EGTA, on the other hand, had no effect on stretch-induced MLC phosphorylation in porcine carotid artery, suggesting that Ca\(^{2+}\) release from intracellular stores sufficiently activated MLC kinase in this tissue. Similarly, treatment with verapamil (200 μmol/L), a VDCC antagonist, had no effect on stretch-induced MLC phosphorylation.

Phosphopeptide map analysis has revealed that stretch results in the incorporation of [32P]phosphate at sites on MLC catalyzed exclusively by MLC kinase; phosphorylation of those sites consistent with PKC activation was not detected. This finding appears to conflict with the results from those studies that implicated a role for PKC. Several lines of evidence taken from studies examining the function of PKC during isometric force production and from studies involving isolated proteins indicate that the mechanism by which PKC mediates force maintenance is most likely independent of MLC phosphorylation. On the other hand, it is more likely that the rise in [Ca\(^{2+}\)], initiates Ca\(^{2+}\)-calmodulin–dependent activation of MLC kinase, and MLC phosphorylation predominantly reflects this activity.

**Overview of Mechanisms Contributing to Myogenic Activity**

As discussed, current evidence suggests that multiple transduction pathways are involved in the regulation of myogenic activity, although the extent to which these mechanisms act singly or together remains to be determined. Those pathways believed to participate in myogenic activity are summarized in the figure. The summary does not attempt to distinguish between the various types of myogenic phenomena nor does it include descriptions of possible temporal differences in the involvement of the various pathways.

In this model, membrane deformation imposed by stretch activates SAC, which permit the entry of Na\(^{+}\) and Ca\(^{2+}\) into the cytosol. Current evidence suggests that SAC density and Ca\(^{2+}\) conductance may not be sufficient to provide enough [Ca\(^{2+}\)] to support sustained vasoconstriction. However, it has been proposed that membrane depolarization due to cation influx through SAC could provide the potential change necessary for increasing the open-state probability of VDCC, thereby augmenting Ca\(^{2+}\) levels sufficient to support vasoconstriction. In opposition to a positive feedback cycle in which Ca\(^{2+}\) influx through VDCC causes progressive membrane depolarization, the opening of K\(_{c}\) in response to both a decrease in membrane potential and an increase in [Ca\(^{2+}\)], would increase the efflux of K\(^{+}\). In effect, this hyperpolarizing current would act as part of a negative feedback loop to limit membrane depolarization during the response to stretch. Finally, the increase in [Ca\(^{2+}\)], would activate MLC kinase, leading to phosphorylation of MLC and accelerated myosin ATPase activity.

In addition to modulation of membrane ion conductances, recent studies implicate G protein activation, either G\(_{i}\) or G\(_{q}\), in response to stretch of vascular smooth muscle membrane or to changes in intravascular pressure. Activation of the G\(_{i}\) protein would result in the inhibition of cAMP production by adenylate cyclase, thereby removing the vasodilator effect of the cyclic nucleotide. Alternatively, the G\(_{q}\) protein may be acti-
Schematic diagram representing possible pathways involved in the transduction of increased intravascular pressure or stretch into the development of myogenic tone or myogenic response. G_i indicates G protein that inhibits adenylate cyclase; G_q, phospholipase C-coupled G protein; SAC, stretch-activated channels; AC, adenylate cyclase; PLC, phospholipase C; V_m, membrane potential; cAMP, cyclic AMP; DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate; VDCC, voltage-dependent Ca^{2+} channels; PKC, protein kinase C; [Ca^{2+}], intracellular Ca^{2+} concentration; MLCK, Ca^{2+}-calmodulin-dependent myosin light chain kinase; K_{Ca}, large-conductance Ca^{2+}-activated K^+ channel; and LC_{20}, phosphorylated 20-kd myosin light chain.

**Implications Relating Myogenic Activity to the Pathogenesis of Hypertension**

Within the cardiovascular system, homeostatic mechanisms function to regulate peripheral vascular resistance to control mean arterial pressure. On a moment-to-moment basis, vascular resistance is determined by the complex interaction of multiple factors, among which are neural, humoral, and local autoregulatory influences. The extent to which these interactions are important in determining vascular resistance is beginning to become more clearly understood as a result of quantitative studies describing some of these interactive relations involving autoregulatory mechanisms.

The potential for autoregulatory mechanisms to contribute to increased vascular resistance has been recognized for several years.114-116 Of particular relevance to this review have been studies demonstrating that pressure-dependent mechanisms of autoregulation can significantly contribute to increases in vascular resistance during hypertension.119-122 The evidence for this is based on an experimental paradigm that involves measuring and comparing changes in regional or whole-body vascular resistance during hypertension, followed by periods during which intravascular pressure is selectively normalized. Under these experimental conditions, normalizing intravascular pressure has been shown to result in a reduction in vascular resistance that reflects the contribution of local mechanisms of blood flow autoregulation. For example, protection of the splanchnic circulation from the elevated systemic arterial pressure that occurs during acute renal hypertension by inflating a protective cuff surrounding the proximal portion of the superior mesenteric artery has been shown to attenuate the rise in superior mesenteric artery resistance by approximately two thirds.120 The pressure-dependent nature of these changes in vascular resistance has suggested that the myogenic mechanism is most likely responsible.

There are several conceivable ways that increases in myogenic activation may contribute to increased vascular resistance in hypertension. As described above, one possibility could involve the acute myogenic response to increases in intravascular pressure. Such short-term adjustments in myogenic activation could lead to vasconstriction, which would secondarily amplify increases in vascular resistance.119-123 A second mechanism could involve enhanced myogenic responsiveness in response to circulating vasoconstrictors such as norepinephrine. For example, α-adrenergic stimulation has been demonstrated to enhance myogenic reactivity in skeletal muscle arterioles from normotensive rats.64-68 A third possibility is that during the pathogenesis of hypertension, an increase in myogenic reactivity may occur.100 This increased reactivity might result from restructuring of the vascular wall or from intrinsic alterations in the sensitivity of vascular smooth muscle to stretch. Whether these possible mechanisms act singly or in combination depending on the stage and type of hypertension has not yet been resolved. They do, however, illustrate the various ways this local mechanism of vasoregulation could contribute to the increases in peripheral vascular resistance that occur in arterial hypertension.

**References**


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