Protein Kinase C Modulates Basal Myogenic Tone in Resistance Arteries From the Cerebral Circulation

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The objective of this study was to determine whether myogenic tone in the cerebral circulation can be modified by agents that interact with protein kinase C (PKC), a modulator of intracellular calcium sensitivity. Pial arteries (194±8 μm at 125 mm Hg) were isolated from Wistar-Kyoto rats and mounted on glass microcannulas in a specialized arteriograph. Simultaneous recordings of transmural pressure and lumen diameter were made with a video-electronic system. Myogenic tone, which developed at transmural pressures above 50 mm Hg, reduced lumen diameter by 29±3%, to 136±5 μm. Staurosporine (a PKC inhibitor) or indolactam (a PKC activator) was added cumulatively to segments of arteries obtained from each animal. Staurosporine induced progressive and eventually complete dilation, with half-maximal inhibition of myogenic tone occurring at a concentration of 1.32±0.10 nM. Conversely, indolactam augmented basal tone, reducing diameter by a maximum of 62±3%, with half-maximal effects at 0.4±1.0 μM. The effects of indolactam on arterial responses to acute increases in transmural pressure were also determined to test whether this dynamic and possibly separate mechanism could be potentiated by PKC stimulation. Although basal tone was augmented, diameter responses to increased pressure were not altered. In summary, these results implicate PKC in the regulation of basal myogenic tone and resistance artery caliber, which is a major determinant of blood flow. PKC modulation did not affect diameter responses to sudden changes in transmural pressure, however, suggesting the existence of a separate sensing/transduction mechanism that has yet to be identified. (Circulation Research 1991; 68:359–367)

Basal myogenic tone plays an important role in the physiological regulation of blood flow. When vascular smooth muscle is maintained in a state of partial activation, arterial diameter can be made to either increase or decrease, thereby facilitating blood flow regulation in a bidirectional fashion.1 Observed most often in smaller arteries and arterioles, this state of maintained constriction is inherently dynamic, because the level of tone can be augmented by increasing pressure or stretch and thereby contribute to the autoregulation of blood flow.2-4 The cellular pathways underlying intrinsic tone are not well understood and involve transduction of physical forces into contractile filament activation through mechanisms that are dependent on the influx of extracellular calcium.5 Considerably more information is available on the mechanisms by which smooth muscle cell activation can occur as a result of extrinsic (receptor-mediated) stimuli.

Initial studies demonstrated that agonist-induced tone is regulated by myosin light chain kinase (MLCK), whose activity is governed by a calcium-calmodulin–mediated phosphorylation.6-7 Unsuccessful attempts to correlate the level of MLCK activity with maintained force production and levels of intracellular calcium have led to the proposal of a latch bridge theory of vascular excitation–contraction coupling. An alternate hypothesis suggests the participation of a non-MLCK–regulated system, having a high affinity for calcium. One such intracellular regulator of calcium sensitivity in a variety of cell types is protein kinase C (PKC).

Activation of PKC after receptor stimulation is thought to occur via activation of receptor-coupled G proteins, which initiate phospholipase C–mediated formation of diacylglycerol and inositol 1,4,5-trisphosphate.8 Inositol trisphosphate diffuses through the cytosol and is a potent stimulus for the release of

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calcium from intracellular stores. Diacylglycerol remains membrane bound and is the primary endogenous activator of PKC, which, in turn, increases the sensitivity of a number of calcium-dependent cellular processes, including those that regulate muscle contraction. However, direct evidence implicating PKC in the regulation of vascular tone, especially under physiological conditions, presently is lacking.

The purpose of this study was to test for the involvement of PKC in the maintenance of basal myogenic tone and to determine whether the efficiency of autoregulatory constriction to increasing transmural pressure can be amplified by pharmacological activation of PKC. In recent years, investigations into the cellular functions of PKC have been facilitated by the development of several compounds that either activate or inhibit this enzyme. In this study, PKC activation was accomplished by using (-)-indolactam V, a synthetic alkaloid tumor promoter with properties similar to phorbol esters. Stauosporine, a microbial product that competes for the ATP binding site on PKC and is reported to be the most potent and selective inhibitor of the enzyme, also was used. Although the PKC system is involved in stretch-induced responses in larger arteries and veins, to our knowledge, this is the first report that implicates PKC activity in the regulation of resistance artery tone and lumen diameter.

Materials and Methods
Preparation of Vessels
Cerebral and mesenteric resistance arteries were obtained from adult (16–24-week-old) male normotensive Wistar-Kyoto rats (n=17). The animals were lightly anesthetized with ether and killed by decapitation; the entire brain was removed immediately and immersed in a dissection dish containing cold oxygenated physiological saline solution (PSS). A tertiary branch of the posterior cerebral artery 0.5–1 mm long then was carefully dissected from each hemisphere and transferred to the experimental chamber of a specialized arteriograph also filled with oxygenated PSS. In some animals (n=12), the abdominal cavity was opened, and a segment of the small intestine 5–6 cm distal to the pylorus was removed for dissection of comparably sized vessels from the mesenteric arcade.

The arteriograph consists of a 20-ml vessel chamber with inlet and outlet ports for superfusing PSS and an optical window in the bottom to facilitate vessel transillumination and visualization with an inverted microscope. Two glass microcannulas (80-μm o.d.) are suspended within the chamber, and one is mounted on a bulkhead attached to a digital micrometer to permit adjustments in axial length.

By using extra fine-point No. 5 microforceps, the proximal end of a vessel segment was cannulated and secured on one cannula with single strands (20-μm diameter) teased apart from a 1-cm length of surgical braided nylon suture. After the lumen was flushed gently with oxygenated PSS, the distal end of each vessel was tied onto the second cannula, and the intraluminal pressure was set to 25 mm Hg with a pressure servo system described below.

The solution in the vessel chamber of the arteriograph was recirculated continuously from a 500-ml external reservoir of PSS at a flow of 20–30 ml/min. The PSS was bubbled with a mixture of 10% O₂, 85% N₂O, and 5% CO₂. Under these conditions, P₂O₂ averaged 103 mm Hg and PCO₂ 34 mm Hg. The ionic composition of the PSS (mM) was NaCl 119, NaHCO₃ 24, KCl 4.7, KH₂PO₄ 1.18, MgSO₄·7H₂O 1.17, CaCl₂ 1.6, glucose 5.5, and EDTA 0.026. A heating pump connected to a heat exchanger warmed the PSS to 37°C, and a thermistor probe immersed in the bath next to the vessel continuously monitored the temperature, which was recorded throughout the experiment. A micro-pH probe was positioned in the bath and connected to a meter; by adjustment of the reservoir gassing rate, a pH of 7.40±0.03 could be maintained.

The arteriograph containing an artery was placed onto the stage of an inverted microscope with a monochrome video camera attached to a viewing tube and was equilibrated for 60–90 minutes at a transmural pressure of 25 mm Hg. A segment of the cannulated and pressurized artery, visible on a black and white video monitor, was made to intersect perpendicularly the television scan lines by the rotation of the camera about the optical axis of the microscope. When the video signal was processed by an electronic dimension analyzer, analog voltages proportional to the lumen diameter and thickness of the vessel wall were generated, recorded on a strip-chart recorder, and displayed in microns on digital voltmeters. This system operates on a principle first used by Wiederhielm, in which changes in optical contrast are used to trigger pulses that initiate sampling of a voltage ramp. Diameter and wall thickness readings were updated every 17 msec, and the precision of the diameter measurements was within 1%. Transmural pressure was controlled with a pressure servo system that consisted of a controller, motorizer, and pressure transducer. When the threaded shaft of a reversible DC linear motor coupled to a syringe was turned, the intraluminal pressure could be held constant or rapidly changed in a stepwise fashion, as required in the last series of experiments. A diagram showing the peripheral instrumentation associated with the pressurized vessel system is shown in Figure 1. Figure 2 is a photograph of a cannulated, transilluminated cerebral artery pressurized to 50 mm Hg.

Experimental Protocols
After equilibration, transmural pressure was raised in increments of 25 mm Hg to 125 mm Hg. Myogenic tone usually appeared between 50 and 75 mm Hg and developed within several minutes; the degree of constriction was maximal at 125 mm Hg and remained stable over time. Higher transmural pres-
sures (150 or 175 mm Hg) induced unstable fluctuations in diameter, with eventual loss of tone due to forced dilation. Dose–response curves to staurosporine (n=6) or indolactam (n=7) were therefore conducted at 125 mm Hg in cerebral arteries, with the exception of three experiments, in which pressure was increased only from 25 to 40 mm Hg, which is below the threshold for myogenic tone development. These vessels were used to determine the sensitivity of MLCK inhibition to staurosporine by first constricting the arteries with an intermediate concentration of K⁺-PSS (30 mM) and then adding staurosporine in a cumulative fashion.

Because mesenteric vessels do not develop intrinsic tone even at 125 mm Hg, arteries (n=8) were subjected to pressure steps identical to those used on cerebral arteries. In some vessels (n=3), dose–response curves to indolactam were made to determine if a vessel that does not possess myogenic tone will constrict to a PKC activator. Others were preconstricted with 30 mM K⁺-PSS (n=5), which induces a 25–35% decrease in diameter (similar to that seen in

**Figure 1.** Schematic diagram of pressurized vessel system. The intraluminal pressure within a cannulated segment is regulated by a pressure controller that drives a motorizer coupled to a syringe; pressure is measured with a pressure transducer and the signal recorded on a strip-chart recorder. The video image of a transilluminated vessel, obtained by attaching a video camera to a microscope, is processed by the video dimension analyzer, which provides a continuous readout of lumen diameter and wall thickness, which also are recorded. During an experiment, the vessel image is visible on a monochrome TV monitor, and experiments can be recorded on a VCR.

**Figure 2.** Photograph of a cannulated, pressurized rat cerebral artery segment (at 50 mm Hg; lumen diameter=113 μm).
cerebral arteries due to intrinsic tone), and then exposed to increasing doses of staurosporine to determine the sensitivity of MLCK inhibition in a resistance artery at a high transmural pressure.

A separate group of cerebral vessels (n=6) was used to test the effects of PKC activation with indolactam on diameter responses to a rapid step change in transmural pressure from 50 to 75 mm Hg or higher. These arteries were equilibrated as above, pressure increased to 50 mm Hg in increments of 25 mm Hg, and tone allowed to develop. Each vessel then was subjected to two or three cycles of 50 → 75 → 50 mm Hg pressure steps until reproducible responses were observed. Increasing concentrations of indolactam then were given, and the steps were repeated at each dose to determine whether pharmacological activation of PKC potentiates the autoregulatory-type constriction to a pressure step increase.

To determine the extent of maximal constriction, cerebral (n=4) and mesenteric (n=4) arteries were depolarized with a solution containing 125 mM K+ and 5 mM calcium, prepared by substituting KCl for NaCl and increasing the amount of CaCl2 at the beginning of an experiment.

At the conclusion of an experiment, each artery was given papaverine (10^-4 M) to induce complete relaxation, and the pressure steps from 25 to 125 mm Hg or greater were repeated to obtain vessel diameter in the absence of tone (cerebral) or to confirm the absence of tone (mesenteric).

**Solutions and Drugs**

The composition of 1.6 mM calcium-PSS is given above; potassium-PSS (125 mM) was prepared by substituting KCl for NaCl and adding 15 g of sucrose per liter. Intermediate K+ (30 mM) was prepared by proportional (4:1) mixing of PSS with 125 mM K+. Papaverine was purchased from Sigma Chemical Co., St. Louis; indolactam from LC Services Corp., Woburn, Mass.; and staurosporine from Kyowa Hakko USA Inc., New York. Papaverine, PSS, and high-potassium PSS solutions were made weekly; indolactam and staurosporine were prepared daily from powder or a frozen stock solution (2.1 x 10^-3 M, dissolved in dimethyl sulfoxide), respectively.

**Statistical Analysis**

The results shown in the text and figures are presented as mean±SEM (number of vessels). EC50 and IC50 values are expressed as geometric means followed by standard errors, determined from logarithmic values. Differences in diameter responses to changes in transmural pressure before and after the addition of indolactam were compared by using a paired t test; a value of p<0.05 was considered significant.

**Results**

**Diameter Responses of Arteries to Changes in Pressure**

The lumen diameters of cerebral and mesenteric vessels used in this study measured at a transmural pressure of 125 mm Hg are shown in Figure 3. It can be seen that the arterial diameters in papaverine, under fully relaxed and distended conditions, averaged 194±8 and 246±21 μm for cerebral versus mesenteric vessels. When transmural pressure is elevated above 50 mm Hg, cerebral artery diameter decreased because of the development of spontaneous myogenic tone, reducing lumen diameter by 29±3%, to 136±5 μm (at 125 mm Hg).

Once it is present, myogenic tone remains stable for hours. If pressure is changed suddenly, however, a dynamic response in lumen diameter is observed that would favor an autoregulation of blood flow (i.e., constriction to increased and dilation to decreased transmural pressure; see Figure 4 and References 1–3).

![Figure 3. Summary of cerebral (CER.) (n=17) and mesenteric (MES.) (n=12) artery lumen diameters (in micrometers; mean±SEM) at a transmural pressure of 125 mm Hg. Vessel dimensions measured under fully relaxed (10^-4 M papaverine [PAPAV]) or fully constricted (125 mM potassium [K+]) conditions. In physiological saline solution (PSS) (1.6 mM Ca^2+), cerebral but not mesenteric vessels develop an intrinsic myogenic tone that reduces lumen diameter by approximately 30%.](image)

![Figure 4. Tracing showing cerebral artery diameter response to step changes in transmural pressure. After the initial distention following an increase in pressure, the artery constricts and attains a smaller diameter in 3–5 minutes.](image)
Although staurosporine is a highly potent and relatively selective inhibitor of PKC, it also inhibits MLCK at higher concentrations. Therefore, the dose-dependent effects of staurosporine on tone produced by elevated potassium concentrations, which is thought to occur directly by MLCK activation without involving the PKC system, were examined in cerebral and mesenteric arteries as well.

**Inhibition of Potassium Tone by Staurosporine**

To examine the effects of staurosporine on depolarization-induced tone, vessels were examined under two different experimental conditions. First, staurosporine inhibition of potassium-induced tone was studied in cerebral arteries lacking intrinsic tone (n=3). This condition could be achieved by maintaining transmural pressure at 40 mm Hg, which is below the threshold for basal tone development. The concentration of staurosporine required to produce equivalent (50%) inhibition of potassium versus myogenic tone was more than three times higher for the former (4.60±0.35 nM; Figure 7, closed circles).

A second approach was to study the effects of staurosporine on potassium tone in comparably sized vessels from the splanchnic circulation. These vessels normally do not develop an intrinsic myogenic tone, even at high transmural pressures. For these experiments, mesenteric arteries (n=5) were pressurized to 125 mm Hg and activated with 30 mM potassium to induce a level of tone comparable to that of cerebral arteries (25–35% reduction in diameter). Cumulative additions of staurosporine were made, as above, and the results are shown in Figure 7 (open triangles). IC$_{50}$ for staurosporine inhibition of potassium tone in the mesenteric arteries was calculated to be

**Figure 5.** Tracing showing mesenteric artery diameter response to step changes in transmural pressure. Note complete absence of any active reconstriction.

In mesenteric vessels, changes in transmural pressure produce passive diameter responses (Figure 5), although the degree of maximal activation possible with a depolarizing solution of 125 mM K$^+$ is comparable in both types of arteries (Figure 3).

**Inhibition of Myogenic Tone by Staurosporine**

Addition of staurosporine to cerebral arteries at 125 mm Hg (n=6) induced a gradual loss of tone (Figure 6). The loss of tone was dose dependent, with complete inhibition occurring at concentrations above 10$^{-8}$ M (Figure 7; open circles). IC$_{50}$ (concentration required for half-maximal inhibition) was calculated to be 1.32±0.10 nM, which is significantly lower than the IC$_{50}$ for staurosporine inhibition of potassium-induced tone also in cerebral arteries ($p<0.01$).

**Figure 6.** Tracing showing responses of a cerebral artery to indolac-tam (INDOL) and staurosporine (STAURO) at a transmural pressure of 125 mm Hg. INDOL produced rapid constriction, whereas STAURO elicited dilation. Note reduced chart speed during STAURO response.
4.14±0.39 nM. The sensitivity to staurosporine for potassium-induced tone was similar (p>0.05) in mesenteric and cerebral arteries lacking pressure-induced myogenic activity.

**Potentiation of Myogenic Tone by Indolactam**

Having observed selective inhibition of intrinsic tone by a PKC inhibitor, next we sought to determine whether pharmacological activation of the PKC system by indolactam would augment the level of pressure-induced basal tone. Addition of indolactam to pressurized cerebral vessels caused a rapid, stable, and reversible vasoconstriction (Figure 6). To establish the dose dependency of the response, cumulative additions were made in cerebral arteries possessing intrinsic tone at 125 mm Hg (Figure 8). Half-maximal concentration (EC₅₀) was calculated to be 0.4±1.0 μM, and at concentrations above 1 μM, the vessels appeared to be almost fully activated, as evidenced by reductions in lumen diameter in excess of 66%. Preincubation with 1.6 nM staurosporine induced a parallel rightward shift in the dose–response curve for indolactam and increased EC₅₀ for indolactam by 2.1±0.3 times (n=3). Whereas indolactam could produce near-maximal constriction in the presence of 1.6 nM staurosporine, it was unable to overcome the inhibitory effects of higher concentrations (greater than 3 nM).

In mesenteric arteries (n=3), which lack pressure-induced myogenic tone, administration of indolactam (0.01–1 μM or greater) failed to produce any measurable vasoconstriction.

**Effect of Indolactam on Cerebral Artery Diameter Responses to Changes in Pressure**

Blood flow autoregulation, which is well developed in the cerebral vasculature, requires dynamic adjustments in vessel diameter in response to changing transmural pressures. As seen in Figure 4, isolated cerebral arteries respond to a pressure increase with rapid constriction, which would contribute to this phenomenon.

Because all of the experiments described above were made at a fixed transmural pressure, the final series of experiments tested the effects of PKC activation with indolactam on the diameter response to acute changes in pressure. Increasing the transmural pressure from 50 to 75 mm Hg reduced lumen diameter by an average of 1.4±1.9% under control conditions (n=6). The addition of an intermediate concentration (approximately EC₅₀) of indolactam (10⁻⁷ M) significantly reduced lumen diameter (p<0.01) but did not produce any visible potentiation of the myogenic response (Figure 9); in fact, the average diameter was increased (+2.4±3.8%), although this difference was not significant (p>0.05).

**Discussion**

The results of this study demonstrate that the level of basal, myogenic tone present in excised, pressurized cerebral microvessels is profoundly altered by agents that interact with PKC. Although the mechanisms of mechanotransduction involved in the myogenic response to transmural pressure or stretch are not well understood, the results of this study show...
that PKC can modulate the extent of basal tone and thereby control lumen diameter in small arteries.

This conclusion is supported by the following observations: 1) Indolactam, a potent activator of PKC, augmented myogenic tone in cerebral arteries and, at higher concentrations, induced nearly maximal vasoconstriction. 2) In comparably sized mesenteric arteries that do not possess myogenic tone, indolactam was completely without effect. 3) Conversely, staurosporine, an inhibitor of PKC, produced dose-dependent vasodilation and, at concentrations greater than 6 nM, induced a complete loss of intrinsic tone in cerebral arteries. 4) Staurosporine was an extremely potent inhibitor of intrinsic tone, requiring significantly lower concentrations than those required to inhibit a similar degree of tone produced by membrane depolarization with potassium.

A number of studies in different species have established that the cerebral circulation normally operates in a state of partial and sustained constriction. Isolated, pressurized pial arteries develop and maintain a level of tone comparable to that existing in vivo. These in vitro observations suggest that the vascular response to changes in transmural pressure is intrinsic to the cells of the arterial wall and appears to be truly myogenic in nature.

Previous investigations have established that myogenic tone is dependent on the presence of extracellular calcium. It has been proposed that, on entry, calcium interacts with both calmodulin and PKC. Although PKC has been implicated in stretch-induced tone in larger vessels, this is the first report to implicate the PKC mechanism in the regulation of resistance artery diameter, a major determinant of blood flow.

In smooth muscle, contractile force is generated by the interaction of actin with myosin, which is regulated primarily by MLCK, a calcium-calmodulin–dependent enzyme. Investigation of the roles of PKC and MLCK in tissue force production has been made possible by the availability of pharmacological agents that interact with these enzymes. However, the information gained from the use of such compounds is not without inherent drawbacks; compounds such as staurosporine are not completely selective and inhibit several enzyme systems, although the inhibition constants appear to be different for PKC, MLCK, and cAMP- and cGMP-dependent protein kinases.

In the experiments described here, we tried to distinguish between the effects of staurosporine on PKC and MLCK by using this previously reported difference in concentration dependency in conjunction with the observation that potassium depolarization–induced tone occurs through MLCK activation but does not appear to involve PKC.

In cerebral vessels, staurosporine showed a greater selectivity for inhibiting myogenic tone (IC50=1.3 nM) as opposed to inhibition of K+ tone (IC50=4.6 nM), implying differential intracellular mechanisms for the two tone moieties. Recently, we have obtained additional support for the role of PKC in modulating myogenic tone using calphostin, a novel inhibitor of PKC activation that has a significantly greater specificity for PKC. Calphostin produced concentration-dependent loss of myogenic tone with an IC50 of 5 nM. Maximally effective concentrations of calphostin for inhibition of myogenic tone had minimal effect on potassium depolarization–induced vasoconstriction (unpublished observations, 1990). These results are of particular interest because calphostin reportedly binds to the regulatory site of the PKC molecule, whereas staurosporine binds to the catalytic (ATP binding) subunit.

When mesenteric arteries were depolarized with 30 mM potassium at 125 mm Hg, the degree of constriction was approximately equal to that present spontaneously in cerebral arteries pressurized to 125 mm Hg. The sensitivity to staurosporine (IC50=4.1 nM) was similar to that found in brain vessels acti-

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**Figure 9.** Effects of indolactam (Indol) (0.1 μM) on cerebral artery lumen diameter responses to a step increase in transmural pressure from 50 to 75 mm Hg. Before the addition of indolactam (control, closed circles), a 25 mm Hg pressure step produced a slight constriction. After the addition of indolactam (open circles), basal tone increased (approximately 25% reduction in lumen diameter); however, the responses to a change in pressure were comparable (p>0.05, n=6). PSS, physiological saline solution.
vated with 30 mM K⁺ at the lower pressure. We interpret these observations to mean that similar intracellular mechanisms are activated by potassium in the mesenteric arteries at 125 mm Hg and cerebral arteries at 40 mm Hg transmural pressure. The implication of this finding is that the threshold transmural pressure for the myogenic response also regulates endogenous activation of PKC.

In conduit arteries that do not possess intrinsic tone, activation of PKC produces constriction that is much reduced in comparison to that produced by other vasoconstrictors. Both the onset and rate of constriction due to the PKC activators can be enhanced by prior treatment with agents known to promote extracellular calcium entry. In vessels possessing intrinsic myogenic tone, such as microvessels from the rat brain (this study) and the rabbit facial vein, the rapid onset of constriction due to phorbol esters and indolactam most likely is related to the basal entry of calcium promoted by stretch. This proposal is supported by the findings of Forder et al., who were able to enhance the onset of phorbol ester-induced contractions by promoting calcium influx.

PKC can mediate vascular smooth muscle constriction by increasing the calcium sensitivity of a number of calcium-dependent cellular processes. The only known endogenous activator of PKC is diacylglycerol, which is formed from the cleavage of phosphatidylinositol 4,5-bisphosphate (PIP₂) after phospholipase C activation. Current dogma proposes that, after receptor stimulation, intracellular calcium levels increase rapidly and then decline to near basal values in seconds, while contractile force is maintained. PKC activation, possibly by lowering the calcium requirement for contractile protein interaction, provides a postreceptor intracellular mechanism whereby maximal force production could be maintained in the face of low levels of intracellular calcium.

In addition to possessing a basal myogenic tone, pressurized cerebral arteries obtained from several different species respond to sudden changes in transmural pressure with diameter adjustments that favor autoregulation of blood flow. The cellular mechanisms underlying this dynamic response are not well understood and may or may not involve some interaction between endothelial and smooth muscle cell function.

The results of this study support this hypothesis, because PKC activation by indolactam clearly potentiated basal tone at concentrations that had no effect on the diameter responses to acute pressure changes. Although the location of the “pressure sensor” has not been identified, smooth muscle cell depolarization occurs with increasing pressure and may represent an additional, PKC-independent mechanism, which is superimposed on basal tone. The combination of depolarization-induced calcium influx and PKC modulation of intracellular calcium sensitivity may offer an efficient, convergent system for the regulation of resistance artery diameter (Figure 10).

Although it is evident that PKC activation increases basal myogenic tone, its inability to augment the dynamic aspects of myogenic tone cannot be ruled out definitively, because smooth muscle activation and subsequent constriction may itself alter the diameter response to changes in transmural pressure.

The precise mechanisms by which physical forces such as pressure or stretch are transduced into intracellular changes such as PKC activation are not known and await future investigations. Taken in conjunction with other studies that implicate PKC in smooth muscle contraction, however, these observations support a role for PKC in the regulation of vascular tone. Definitive confirmation of a physiological role awaits the development of more specific agonists and antagonists and of biochemical techniques that will afford a better understanding of the complex enzymatic pathways in transmembrane signaling.

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