PROTEIN KINASE C AND SMOOTH MUSCLE CONTRACTION

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SUMMARY

• Protein kinase C (PKC) is a family of phospholipid-dependent Ca\(^{2+}\)/diacylglycerol-stimulated enzymes. It is an ubiquitous protein that plays a multifunctional role in the transduction of extracellular signals activating the phospholipid turnover and generating diacylglycerol (DAG). DAG can be produced from the hydrolysis of phosphatidylinositol-1,4-bisphosphate, phosphatidylcholine, or sphingomyelin by respective phospholipases. PKC is also the major intracellular receptor for phorbol esters, potent tumor promoters, which may replace DAG for its activity. To date, 12 different PKC isoforms have been described, all regulated by phosphatidylserine and DAG, but only some being Ca\(^{2+}\)-dependent (α, β, γ, δ, ε, θ, ι, α, ι, θ, and H) isoforms are Ca\(^{2+}\)-independent. Each isoenzyme is a single polypeptide with four conserved (C1-C4) and five variable (V1-V5) regions. Cl and C2 regions constitute the regulatory domain and contain binding sites for phosphatidylserine, Ca\(^{2+}\), DAG, and phorbol esters, while C3 and C4 constitute the catalytic domain and contain binding sites for ATP, substrates, and various inhibitors. PKC isoenzymes are involved in a variety of cellular responses in eliciting growth, differentiation, neuronal development, synaptic transmission, secretion. They were first implicated in the regulation of smooth muscle contraction with the observation that phorbol esters induce slowly developing, sustained contractions. Both Ca\(^{2+}\)-dependent (α, β) and Ca\(^{2+}\)-independent (ε, θ PKC isoforms have been identified in smooth muscle cells (SMC), and their involvement in the regulation of smooth muscle contractile state has been proved. Different isoenzymes may be activated by different contractile agonists in different SMC types. There are many potential targets for PKC in SMC that could play a role in the modulation of their contractility. Two actin filament-associated proteins, calponin and caldesmon, were shown to be substrates of PKC in SMC. They may regulate the interaction between actin and phosphorylated myosin. The phosphorylated calponin or caldesmon alleviate the inhibition of the crossbridge cycling rate. (Biomed Rev 1997; 8: 87-100)

INTRODUCTION

• The contractile machinery of smooth muscle is under the control of various intracellular messengers and enzyme systems. Most of them, Ca\(^{2+}\)-dependent or Ca\(^{2+}\)-independent, modulate contractility in response to neuroendocrine factors. In this respect, the enzymes of the protein kinase C (PKC) family function as an important feedback system which couples contraction to the overall energy balance of smooth muscle cells (SMC). PKC was first implicated in the regulation of SMC contraction with the observation that phorbol esters induce slowly developing, sustained contractions (1-3).

In this review we shall discuss the properties, isoenzymic forms, subcellular localization, regulation, and translocation of PKC with emphasis on the physiological role of this kinase.
family in the regulation of smooth muscle contraction.

**Ca\(^{2+}\)-CALMODULIN DEPENDENCE OF SMOOTH MUSCLE CONTRACTION**

- It is well known that the primary mechanism in the regulation of smooth muscle contraction involves the myosin phosphorylation/dephosphorylation cycle (Fig. 1). Various extracellular signals cause an increase of intracellular free calcium concentration ([Ca\(^{2+}\)]). Calcium binds to calmodulin (CaM) to form Ca\(^{2+}\)-CaM which can interact with target proteins such as myosin light chain kinase (MLCK). The ternary complex Ca\(^{2+}\)-CaM-MLCK represents the active kinase form (4) that catalyzes phosphorylation of the two 20-kD light chains of myosin. In turn, the phosphorylated myosin can bind to actin, forming acto-P-myosin which triggers crossbridge cycling and development of force at the expense of adenosine triphosphate (ATP) hydrolysis (5). Relaxation generally occurs upon removal of Ca\(^{2+}\) from the cytosol, resulting in dissociation of the Ca\(^{2+}\)-CaM-MLCK complex and regeneration of inactive MLCK (6). Myosin is then dephosphorylated by one or more myosin light chain phosphatases (7).

**PROTEIN KINASE C FAMILY IN SIGNAL TRANSDUCTION**

- PKC belongs to a family of serine/threonine kinases, which has been shown to play a crucial role in signal transduction in response to various stimuli such as growth factors, hormones, neurotransmitters, tumor promoters, thus being involved in the regulation of diverse cellular processes: growth, differentiation, metabolism, secretion, smooth muscle contraction (8-12).

The discovery of the Ca\(^{2+}\)/lipid-dependent PKC in 1977 (13, 14) provided the first indication that the plasma membrane lipids could be recognized as precursors of second messenger molecules generated in the cell upon stimulation of cell surface receptors (14). The best characterized phospholipid pathway as a source of signal molecules is the phosphatidylinositol bisphosphate (PIP\(_2\)) hydrolysis, which generates two second messengers: the water-soluble headgroup, inositol trisphosphate (IP\(_3\)), and the lipid backbone, diacylglycerol (DAG) (16, 17). This hydrolysis requires the activation of phosphoinositide-specific phospholipase C (PI-PLC), which is induced upon binding of an agonist to a specific cell surface receptor. Interaction between the receptor and PI-PLC via a coupling G-protein causes activation of the enzyme (18, 19) (Fig. 2). IP\(_3\) induces Ca\(^{2+}\) release from the intracellular storage pools, which in turn causes translocation of the cytosolic PKC to the plasma membrane, where it is activated by DAG. Activation of PKC requires association of the enzyme with plasma membrane phospholipids, in particular phosphatidylserine (PS) (20, 21). In the absence of metal cations PKC binds PS by electrostatic interactions. The presence of Ca\(^{2+}\) greatly enhances the binding in a concentration-dependent manner (22). It was postulated that Ca\(^{2+}\) may participate in phospholipid clustering (23), and form a bridge between PKC and the acidic lipids (24). The estimated stoichiometry for PKC activation has suggested that for a complete activation of one PKC molecule are required one Ca\(^{2+}\), one DAG, and four PS molecules (25).

In addition to the classical inositol phospholipid turnover pathway, phosphatidylincholine (PC) also serves as a source of DAG (10, 15, 26, 27) (Fig. 3). An agonist-induced activation of phospholipase D (PLD) leads to hydrolysis of PC to phosphatic acid (PA) and choline, PA being subsequently converted to DAG by a PA phosphohydrolase. Although PA is also proposed to be a direct activator of PKC (27, 28), the significance of this acidic lipid as a second messenger remains to be established. Alternatively, PC may be hydrolyzed by a PC-specific phospholipase C (PC-PLC) to form DAG directly (26-28).

DAG molecules generated from PIP\(_2\) by PI-PLC activation undergo fast degradation. However, DAG concentration often increases again with a relatively slow onset, and persists for minutes, occasionally for hours (10). This second wave of DAG is thought to result from the PC hydrolysis, because the fatty acid composition of the secondary DAG matches that of PC. The sustained elevation of DAG is frequently observed in response to long-acting signals such as growth factors, cytokines, and phorbol esters (26, 29, 30). In addition, PKC can also be activated by lysoPC and cis-unsaturated fatty acids, including arachidonic, oleic, and linoleic acids derived from cell membrane phospholipids after their breakdown by phospholipase A\(_2\) (PLA\(_2\)) (15, 31, 32).
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**Figure 1.** Schematic representation of myosin phosphorylation/dephosphorylation as a primary mechanism in regulation of smooth muscle contraction. CaM, calmodulin; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; ATP, adenosine triphosphate; ADP, adenosine diphosphate; P, inorganic phosphate (according to Ref 5).

**Figure 2.** Schematic illustration of signal transduction pathway by inositol phospholipid hydrolysis. PM, plasma membrane; R, receptor; Gp, G-protein; PLC, phospholipase C; PIP₃, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; IP, inositol-1,4,5-trisphosphate; PS, phosphatidylserine.
Figure 3. PKC activation by signal-induced hydrolysis of plasma membrane phospholipids. PIP, phosphatidylinositol 4,5-bisphosphate; PC, phosphatidylcholine; PI-PLC, phosphoinositide-specific phospholipase C; PC-PLC, phosphatidylcholine-specific phospholipase C; PLD, phospholipase D; PLA, phospholipase A₂; SMase, sphingomyelinase IP, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PA, phosphatidic acid; FFA, free fatty acids.

Sphingomyelin (SM) hydrolysis is also recognized as a major phospholipid signalling pathway (33-36). Current results show that the action of a number of extracellular agents (i.e. dihydroxyvitamin D₃, tumor necrosis factors, interleukins, and interferons) results in an early activation of sphingomyelinase (SMase) that cleaves membrane SM (Fig. 3). SM breakdown products such as ceramide have emerged as potential mediators of the effects of these extracellular agents on cell growth, differentiation, and apoptosis (15, 35). Although the mechanisms underlying the action of ceramide are not clear, it appears that this molecule is also involved in the regulation of PKC activity. The action of ceramide on PKC is isoform-dependent, and comprises both direct and indirect effects. It has been shown that ceramide activates PKCα in vitro and in vivo in NIH 3T3 cells (37), and inhibits PKCa translocation to the plasma membrane in both mouse epidermal cells and human skin fibroblasts (38). In addition, PKC is negatively regulated by sphingosine and lysosphingolipids. Sphingosine, the major breakdown product of the sphingolipids, is a potent PKC inhibitor, competitive with respect to binding of DAG and phorbol esters, and noncompetitive in case of Ca²⁺ binding (33-36). Thus, PKC may be physiologically regulated by both positive (DAG) and negative (sphingosine and lysosphingolipids) messengers (35).

Phorbol esters, potent tumor promoters, possess a molecular structure similar to that of DAG, and can substitute DAG in activating PKC (8, 10, 31, 39). Like DAG, they dramatically increase the affinity of PKC for Ca²⁺, resulting in its complete activation at a physiological Ca²⁺ concentration. Since phorbol esters are slowly metabolized, they persist in cells for a much longer times than DAG, and cause a prolonged activation of PKC. Furthermore, they induce an irreversible insertion of PKC into phospholipid vesicles (40), which possibly causes additional perturbation of the cell membrane. The effect of many biological agonists are mimicked by phorbol esters, implicating a role for PKC in the transduction of these signals.

DOMAIN STRUCTURE AND ISOENZYMES OF PROTEIN KINASE C

- It has been established that PKC is not a single
molecule entity, and that many closely related PKC isotypes exist. At present, the mammalian PKC family consists of 12 different isoenzymes, indicated α, β1, β2, γ, λ, μ, σ, ε, θ, ω, and κ (8, 27, 41-43). The members of the PKC family are composed of a single polypeptide with a molecular weight of approximately 80 kDa, with a N-terminal regulatory region (20-40 kDa), and a C-terminal catalytic region (45 kDa). Comparison between the primary structures of PKC isoenzymes from cDNA sequence determinations revealed four conserved (C1-C4), and five variable (VI-V5) regions (Fig. 4). The C1 and C2 regions constitute the regulatory domain and contain binding sites for PS, Ca\(^{2+}\), DAG, phorbol esters (their exact binding sites are not known). The C3 and C4 regions constitute the catalytic domain and contain binding sites for ATP, PKC substrates, and various inhibitors (44-47).

The C1 domain contains a pseudosubstrate (autoinhibitory) sequence resembling a PKC consensus phosphorylation site (48) with an alanine for serine/threonine substitution. This domain also contains two cysteine-rich,", zinc butterfly" motifs (designated CYST1 and CYST2), responsible for DAG and phorbol ester binding (49). The pseudosubstrate domain has been proposed to bind to the active site and maintain the kinase in an inactive state by steric inhibition. Binding of activators is thought to induce a conformational change that displaces the pseudosubstrate (47, 48). The C2 domain contains the Ca\(^{2+}\)-binding site (50), and deletion of this domain results in loss of the Ca\(^{2+}\) requirement for kinase activity (51). The regulatory and catalytic parts are separated by a hinge (V3) region that becomes proteolytically labile when the enzyme is bound to the membrane. A proteolytically generated kinase domain (protein kinase M), free of pseudosubstrate inhibition, is constitutively active (13).

Based on their structure and cofactor requirement, PKC isoenzymes can be divided into three groups: classical or conventional (cPKC), novel (nPKC), and atypical (aPKC) (8, 10). All members of the PKC family require PS for their activation. The best characterized and first discovered are cPKC: α, two alternatively spliced variants βI and (βII, and γ. These isoenzymes are strictly Ca\(^{2+}\)-dependent. The next well characterized arePKC: ε, θ, γ, and η. These isoenzymes are structurally similar to cPKC, but lack the C2 domain and exhibit kinase activity that is Ca\(^{2+}\)-independent. The least understood are αPKC: ε and η. These differ significantly in structure - their C2 domain is absent (i.e. they are Ca\(^{2+}\)-independent), their C1 domain contains only one Cys-rich motif (not two), and furthermore, they have been reported not to respond to phorbol esters (10, 47).

PKCa is present in most cell types, whereas the other isofoms appear to be expressed only in specific tissues. Multiple isoforms may coexist in the same cell type (8, 10, 41). Differences in the subcellular localization of PKC isotypes have been also reported. Their compartmentation appears to be dynamic, and can change depending on the activation state of the cell. Recently, detailed studies using isolated subcellular structures or in situ immunocytochemical analysis have determined that in addition to the plasma membrane, PKC isoenzymes also translocate to the cytoskeleton, Golgi apparatus, endoplasmic reticulum, perinucleus, and nucleus (52, 53). It has been assumed that PKC may also bind to specific targeting proteins located at various subcellular sites, presumably in proximity to potential substrates. Using a PKC overlay assay with a cytoskeletal protein fraction and detection with anti-PKC(3, a class of PKC binding proteins termed receptors for activated C-kinase has been identified that binds to PKC only in the presence of PS, DAG, and Ca\(^{2+}\) (54). When PKC becomes activated, it causes phosphorylation of substrate proteins and undergoes autophosphorylation taking place at six basic amino acid residues in three regions: the N-terminal
peptide, the C-terminal tail, and the hinge region between the regulatory and catalytic domain (55). Several lines of evidence suggest that PKC autophosphorylation increases the affinity of the enzyme for phorbol esters and Ca\(^{2+}\) (56), and accomplished at distinct sites may cause distinct types of enzyme regulation (55).

PROTEIN KINASE C IN THE REGULATION OF SMOOTH MUSCLE CONTRACTION

- The potential involvement of PKC in the regulation of the contractile state of vascular smooth muscle was originally suggested by the observation that phorbol esters, which specifically activate PKC (57), induce slow, sustained contractions of a variety of vascular smooth muscle strips (1-3, 58). In some instances, this results from an increase of \([\text{Ca}^{2+}]_{i}\) with activation of MLCK, phosphorylation of the 20-kDa light chains of myosin (LC\(_20\)), and consequently, force development (5). In other cases, however, phorbol ester-induced contractions occurred without a change in \([\text{Ca}^{2+}]_{i}\) or an increase in myosin LC\(_{20}\) phosphorylation (3, 58-60).

The mechanism of activation of SMC contractions by phorbol esters shows a considerable tissue specificity, and distinct PKC isoforms are engaged in different cell types upon action of different stimuli. Investigations using swine carotid artery have shown that phorbol 12,13-dibutyrate (PDB) increased the \(\text{Ca}^{2+}\) and phospholipid-dependent PKC activity in isometrically contracting arterial smooth muscle strips with a time course which paralleled or preceded force development. The contractile agonist histamine had the same effect. Arterial SMC express both PKCa and PKC\(_{3}\) as principal isoenzymes (61). In ferret portal vein, PKCa has also been implicated in the regulation of contraction (62), and this tissue displays a totally \(\text{Ca}^{2+}\) dependent contraction (52). On the other hand, PKC-activating 12-deoxyphorbol 13-isobutyrate 20-acetate (DPBA) was shown to elicit a slow, sustained contractile response in ferret aortic strips (3), which was observed in the absence of extracellular \(\text{Ca}^{2+}\). No change in \([\text{Ca}^{2+}]_{i}\) was detected with the photoprotein aequorin, and quantification of LC\(_{0}\) phosphorylation revealed no change in the resting level of myosin phosphorylation (3, 58). These studies suggest that proteins other than myosin, phosphorylated by PKC, may also be involved in regulation of SMC contraction. Furthermore, activation of PKC resulted in a contractile response to \(\text{Ca}^{2+}\) at resting \([\text{Ca}^{2+}]_{i}\) (220 nM), meaning that phorbol ester-induced contraction was \(\text{Ca}^{2+}\)-independent (3). To test the hypothesis that PKC was indeed involved in the contractile response of the ferret aorta to phorbol esters, saponin-permeabilized single-cell preparations were used. The advantage of this system is that it retains the contractile machinery functionally intact, and permits clamping of \([\text{Ca}^{2+}]_{i}\), and addition of large molecules such as peptides and enzymes, which thereby have access to the contractile proteins. Saponin-permeabilized single cells retained endogenous PKC since they responded to DPBA similarly to the intact cells. They also retained receptor coupling, i.e., the ability to contract in response to oc-adrenergic stimulation. At pCa 7.0 (100 nM \(\text{Ca}^{2+}\), close to the resting \([\text{Ca}^{2+}]_{i}\)), phenylephrine elicited a significant force development, suggesting \(\text{Ca}^{2+}\) sensitization of the contractile response. However, the phenylephrine-induced contraction still persisted at [Ca\(^{2+}\)], close to zero (pCa 8.6), which suggests that phenylephrine triggers a \(\text{Ca}^{2+}\)-independent pathway, and PKC-mediated contraction is \(\text{Ca}^{2+}\)-independent. The involvement of PKC in triggering contraction was confirmed by the addition of protein kinase M, the constitutively active catalytic fragment of PKC generated by trypsin digestion. The advantage of protein kinase M over PKC is that phosphorylation of PKC substrates can be achieved in the absence of cofactors (\(\text{Ca}^{2+}\), phospholipids, DAG). Supplementation of protein kinase M to the medium bathing the permeabilized single cells resulted in a slow, sustained contraction similar to that induced in intact cells by phenylephrine (63). Other results also suggest the involvement of \(\text{Ca}^{2+}\)-independent PKC in oxytocin-induced contractions of rat uterine SMC in the absence of extracellular \(\text{Ca}^{2+}\) (64). Such contractions occurred without a change in \([\text{Ca}^{2+}]_{i}\), and in the absence of myosin LC\(_{20}\) phosphorylation. They were inhibited by the selective PKC inhibitors staurosporine and H-7 (65). Prostaglandin F\(_{2}\), was also found to cause a \(\text{Ca}^{2+}\)-independent contraction of permeabilized ferret aortic SMC, and this contraction was due, at least in part, to PKC activation, because it could be inhibited by the PKC pseudosubstrate peptide inhibitor and staurosporine, again implicating a \(\text{Ca}^{2+}\)-independent PKC isoenzyme(s) involvement (66).

The expression of \(\text{Ca}^{2+}\) independent PKC isoenzymes in ferret aortic smooth muscle has been investigated by Western blotting using isoenzyme-specific antibodies (52, 67). Only PKC\(_{e}\) and PKC\(_{t}\); have been detected, suggesting that these isoenzymes may be responsible for a \(\text{Ca}^{2+}\)-independent contraction of the ferret aorta. Since PKCe but not PKCt; is responsive to phorbol esters, the former seems more likely to be involved in phorbol ester-induced contraction. Furthermore, upon stimulation of ferret aortic SMC with phenylephrine, PKCe translocated from the sarcoplasm to the sarcolemma, whereas PKC\(_{t}\) translocated from a perinuclear localization to into the nucleus (52). Horowitz et al (68) purified PKCe and PKCt; free of other PKC isoenzymes, and examined their effects on permeabilized single ferret aortic SMC. They found that PKCe induced a slow, sustained contraction, similar to that caused by phenylephrine, that was reversed by the PKC pseudosubstrate peptide inhibitor. PKCe on the other hand, failed to elicit a contractile response (68). These results support the hypothesis that the \(\text{Ca}^{2+}\)-independent contraction
initiated by phenylephrine orphorbol ester is mediated by PKCe.

**POtential Substrates of Protein Kinase C in Vascular Smooth Muscle**

- A great deal of efforts has been devoted to identifying the substrate(s) of PKC involved in the contractile responses. The weight of evidence is against either myosin or MLCK being involved (67, and Refs therein). Probably of importance is PKC-catalyzed phosphorylation of proteins responsible for Ca\(^{2+}\) flux across the sarcolemma or sarcoplasmic reticulum membrane, resulting in [Ca\(^{2+}\)]. increase. In the cases of phorbol ester-induced contractions of vascular SMC that do not involve a [Ca\(^{2+}\)], increase or LC\(_{20}\) phosphorylation, it is necessary to look for other PKC substrates. Based largely on two-dimensional gel electrophoresis of proteins from \(^{32}\)P-labelled tissue treated with phorbol esters or various agonists, and in vitro phosphorylation of SMC proteins by purified PKC, the actin filament-associated proteins calponin (69-72), and caldesmon (5, 11, 73-75) have been implicated in the regulation of smooth muscle contraction.

Calponin is an actin- and CaM-binding protein found exclusively in smooth muscle. It inhibits SMC actomyosin ATPase through its interaction with actin. Calponin can be phosphorylated by PKC or CaM kinase II, suspending its inhibition on the actomyosin ATPase activity (71). Calponin was found to be in vitro substrate of Ca\(^{2+}\)-dependent PKC (a mixture of a, P and y isoenzymes) (76), a very good substrate of PKCe, but a poor one of PKC\(^\alpha\) (77). The principal site of PKC phosphorylation was identified as serine 175, with substoichiometric phosphorylation at several other sites, including threonine 184 (72). Recent characterization of site-specific calponin mutants, in which serine 175 was replaced by alanine, aspartic acid or threonine suggested a key role for this residue in binding to actin, and the subsequent inhibition of actomyosin ATPase (78). Dephosphorylation of calponin by a protein phosphatase (identified as type 2A) restored actin binding and actomyosin ATPase inhibition (79). The participation of calponin in SMC contraction was confirmed in experiments with permeabilized ferret aortic cells (80). Addition of calponin to these cells, maximally contracted with phenylephrine or PKCe, resulted in about 50% reduction of steady-state force. When calponin was applied prior to PKCe, contraction was completely suppressed. Phosphorylated calponin, however, had no effect on contractile force; neither did a site-specific calponin mutant, in which the principal phosphorylation site (serine 175) was changed to alanine.

The possible mechanisms of regulation of cross-bridge cycling in SMC by calponin are presented in Fig. 5. Extracellular signals such as a,-adrenergic agonists can trigger DAG pro-

**Indirect Effects of Protein Kinase C on Smooth Muscle Contraction**

- Despite proteins involved in the regulation of contraction, several other proteins were found to be targets of

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Figure 5. Possible signalling pathways describing the regulation of smooth muscle contraction by phosphorylation of calponin. PI-PLC, phosphoinositide-specific phospholipase C; PLD, phospholipase D; PLC, phospholipase C; PC, phosphatidylcholine; PIP, phosphatidylinositol 4,5-bisphosphate; PA, phosphatidic acid; IP, inositol 1,4,5-trisphosphate; PA-PHA, phosphatidate phosphohydrolase; DAG, diacylglycerol; CaP, calponin; P-CaP, phosphorylated calponin; PP2A, type 2A protein phosphatase; PP1M, myosin light chain phosphatase.
PKC-induced phosphorylation, some of them related to intracellular Ca^{2+} homeostasis, and cell membrane excitability. Recent studies have suggested that PKC may be stimulated also by processes independent of translocation. A pool of PKC resident in native membranes of various cells has been revealed, that can be directly stimulated by DAG or phorbol esters at very low concentrations (89). This finding raises the question about possible targets of the constantly membrane-bound forms of PKC. The most prominent candidates are the membrane ion channels.

Several studies have proved that PKC phosphorylate ion channels in SMC. Thus, the most widely expressed member of K^+ channel family in mammalian SMC, Kv_{16}, was shown to be in vivo phosphorylated by PKC, leading to a decrease of the channel opening probability (90, 91). Phorbol esters were also found to suppress the ATP-sensitive K⁺ currents (92, 93) in guinea pig visceral SMC, and to slow down the inactivation of L-type Ca^{2+} current (94, 95). Inhibition of basal PKC activity by selective inhibitors was found to result in more than two-fold increase of the Ca^{2+}-sensitive K⁺ conductance in guinea pig ileum and rat tail artery (Durananova et al, unpublished data), as well as to cause relaxation of guinea pig stomach antrum (96). In all these cases, the activation of PKC resulted in cell membrane depolarization, activation of Ca^{2+} entry, and increased cell excitability upon conventional stimuli. However, in certain SMC, phorbol esters were shown to suppress the voltage-sensitive Ca^{2+} entry (97, 98) or to attenuate the intracellular Ca^{2+} release (99). Controversial effects of phorbol esters have been observed in different types of SMC depending on their preexposure to various agonists (100, 101). These data suggest that PKC-dependent modulation of ion channel activity may be an integrative consequence of various intracellular events comprising a cross-talk between two or more second messenger signalling pathways.

**CONCLUSION**

- The studies summarized above support the concept that other mechanisms, in addition to Ca^{2+}/CaM-dependent activation of MLCK and phosphorylation of LC_{20}, are involved in the regulation of smooth muscle contractile activity. Activation of PKC has been proposed to be an important pathway for regulating smooth muscle contraction. PKC is an integral part of the cell signalling machinery. Like most other signalling proteins, this enzyme comprises a large family with multiple isoforms exhibiting individual characteristics and distinct patterns of tissue distribution. The biological significance of this heterogeneity has not been fully clarified, but the function of each PKC isoform in cell regulation is being investigated extensively. Both Ca^{2+}-dependent (α, p) and Ca^{2+}-independent (γ, Q PKC isoenzymes have been identified in SMC, and they undergo differential activation in different SMC types upon different stimuli. The coupling between PKC activation and its biological effects is mediated by various protein substrates, which become phosphorylated in response to agonist stimulation. Calponin, a smooth muscle actin filament-associated protein, has been implicated in the regulation of SMC contraction as a substrate of PKC. Recent evidence suggests that PKC may trigger a cascade of phosphorylation reactions, resulting in phosphorylation of another actin filament-associated protein, caldesmon. However, evaluation of the importance of PKC-catalyzed phosphorylation of calponin and caldesmon in intact muscles requires further experimentation. Much work remains to be done to elucidate the mechanisms by which SMC contractions are mediated by members of PKC family via distinct substrate phosphorylation. Future studies on the regulation of smooth muscle contraction will
focus on the molecular details of the signal transduction pathways, and the cross-talk between them.

ACKNOWLEDGEMENTS

- This work was supported by The National Science Fund, grant No. K606-96. We thank the Physiological Society of UK for providing experimental equipment for patch-clamp studies.

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