Protein kinase Cα participates in activation of store-operated Ca\(^{2+}\) channels in human glomerular mesangial cells

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Received 14 June 2002; accepted in final form 24 June 2002

Abstract


Protein kinase C (PKC) is composed of a family of related isoenzymes, grouped into three major classes of conventional Ca\(^{2+}\)-dependent PKCs (α, βI, βII, and γ), novel Ca\(^{2+}\)-independent PKCs (δ, η, θ, and ε), and atypical Ca\(^{2+}\)-and lipid-independent PKCs (λ, ζ, μ, and η) (6, 35, 36). All isoforms express distinct enzymological properties, differential tissue distribution, different substrate specificity, and specific subcellular localization with distinct modes of cellular regulation (4, 6, 9, 18, 23, 36, 38). For example, PKCα, δ, ε, and ζ, but not PKCβII, which is strongly expressed in cardiomyocytes, were detected in rat MC as determined by Western blotting (18, 19, 42). In renal epithelial cells, PKCβI, ε, and α are all localized in the cytoskeletal compartment; however, only PKCβII and α are able to translocate from the cytosol to membranes on activation by the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA; Refs. 4 and 34). Moreover, PKCα is a positive mediator of vascular smooth muscle proliferation (37), whereas PKCβII is inhibitory (50). Whereas PKCα promotes cell growth in vascular smooth muscle, PKCα depresses proliferation of a human colonic adenocarcinoma cell line (43). These differences in structure, enzymatic properties, and intracellular localization illustrate that each of the PKC isoforms possess specific cellular functions.

Previous studies from this laboratory have demonstrated that PKC mediates epidermal growth factor and thapsigargin-induced activation of SOC via a phosphorylation mechanism, measured by fura 2 fluorescence and patch clamping (26, 27). The present study was performed to determine which specific isofrom of PKC is the intermediary messenger in this signaling pathway. Fura 2 fluorescence and conventional patch clamping were combined with biochemical approaches to examine the involvement of the classic isoforms PKCα, βI, βII, and γ. Because obtaining whole cell currents is technically difficult in MC, single-channel current recordings and whole cell Ca\(^{2+}\) measurements with fluorescent dyes were employed in the present study.
EXPERIMENTAL PROCEDURES

Preparation of Cultures of MC

The details regarding the procedures and methods for culturing MC were described in a previous study (13). Briefly, MC were purchased from Biowhittaker (Walkersville, MD) and cultured in Dulbecco’s modified Eagle’s medium (DMEM; Sigma Chemical, St. Louis, MO) supplemented with 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 2.0 mM glutamine, 0.66 U/ml insulin, 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20% fetal bovine serum (pH 7.2–7.4). Only subpassages of MC ≤11 generations were used. Upon achieving confluence, cells were passed onto 22 × 22–1 mm coverslips (Fisher, Pittsburgh, PA) and studied within 56 h. The cover slips served as the floor of a perfusion chamber (Warner RC-2OH, 23°C) used in both fura 2 and patch-clamp experiments.

Measurement of [Ca2+]i

The intracellular Ca2+ concentration [Ca2+]i, was monitored in MC using fura 2 and dual excitation wavelength fluorescence microscopy, as previously described (3, 12). In brief, MC were incubated with physiological saline solution containing 7 μM fura 2-AM, 0.09 g/dl DMSO, and 0.018 g/dl Pluronic F-127 (Molecular Probes, Eugene, OR) for 60 min at 23°C. A selected individual cell was illuminated alternately at excitation wavelengths of 340 and 380 nm (bandwidth = 3 nm) provided by a DeltaScan dual monochromator system (Photon Technology International, Monmouth Junction, NJ). The emission wavelength was 510 nm. Background-corrected images were recorded using the FeliX software package (Photon Technologies). Calibration of the fura 2 signal was performed according to the manufacturer’s instructions for a classic PKC isoform at an appropriate dilution (1:50). A horseradish peroxidase-labeled goat anti-rabbit or anti-mouse IgG secondary antibody was then used to react with PKC antibodies at 1:50,000 dilution. The immunoblots were labeled by enhanced chemiluminescent (ECL) reagents and then placed against autoradiography film and developed in a Kodak M35A X-Omat processor. The isoforms of PKC in cytosolic and membrane fractions were quantified by measuring densitometry of specific bands using Quantity One 4.1 software. In each group, the total optical densities of a PKC isoform in the cytosolic and membrane fractions were counted as 100%. The amount of individual isoform in either fraction was expressed as a percentage of the total optical density.

PKCα Antisense Oligonucleotide Treatment

Translation of PKCα RNA was inhibited by using a phosphorothioated PKCα antisense oligonucleotide (made in the molecular core lab of the Eppley Institute of the University of Nebraska Medical Center, Omaha, NE) complementary to a region from the initiation codon of PKCα (nucleotide 495–TAC CGA CTG CAA AAG GCC CCG-3′ nucleotide 28). The control was the scrambled nonsense oligonucleotide (5′-GCA TAG TCA TGG CCT TTA AAT). A stock oligonucleotide solution (2.5 μM) was diluted 1,000 times with DMEM supplemented with 20% FBS to a final concentration of 2.5 nM. MC were incubated with the oligonucleotide containing medium for 24–48 h at 37°C before experimentation.

Solutions and Chemicals

For all fura 2 and cell-attached patch experiments, the initial extracellular physiological saline solution (PSS) contained (in mM): 135 NaCl, 5 KCl, 10 HEPES, 2 MgCl2, and 1 CaCl2. For inside-out patches, the bathing solution contained (in mM): 140 KCl, 2 MgCl2, 0.001 CaCl2, and 10 HEPES. The pipette solution for all patch experiments contained 90 mM NaCl, 2 mM MgCl2, 0.001 CaCl2, 10 mM glucose, 1 mM HEPES, 0.5 mM EGTA, and 0.5 mM tetraethylammonium (TEA). A horseradish peroxidase-labeled antibody against PKCα (made in the molecular core lab of the Eppley Institute of the University of Nebraska Medical Center, Omaha, NE) and a nonhuman polyclonal antibody against receptor-operated channels were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The control was the scrambled nonsense oligonucleotide complementary to a region from the initiation codon of PKCα (nucleotide 495–TAC CGA CTG CAA AAG GCC CCG-3′ nucleotide 28). The control was the scrambled nonsense oligonucleotide (5′-GCA TAG TCA TGG CCT TTA AAT). A stock oligonucleotide solution (2.5 μM) was diluted 1,000 times with DMEM supplemented with 20% FBS to a final concentration of 2.5 nM. MC were incubated with the oligonucleotide containing medium for 24–48 h at 37°C before experimentation.
methyl ether (HBDDE), purified PKCa, βI, βII, γ, ATP, and specific primary antibodies to PKCa, βI, βII, and γ were purchased from CalBiochem (La Jolla, CA). LY-379196 was obtained from Eli Lilly (Indianapolis, IN). The secondary antibodies were purchased from Jackson ImmunoResearch Lab (West Groba, PA).

**Statistical Analysis**

In patch-clamp experiments, all NP values were calculated from at least 10 s of single-channel recording. Comparisons between two individual groups were performed by using a Student t-test. One-way ANOVA, followed by Student-Newman-Keuls tests were used for comparisons among multiple groups. Data are reported as means ± SE; n is the number of cells. Significance was P < 0.05. Statistical analysis was performed using SigmaStat (Jandel Scientific, San Rafael, CA).

**RESULTS**

**Fura 2 Experiments**

Effects of specific PKC isoform inhibitors on thapsigargin-induced capacitative Ca\(^{2+}\) entry. Thapsigargin, a specific inhibitor of the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) (45), has been used as an efficient tool to specifically activate SOC in a variety of cell types (40). Using fura 2 fluorescence ratiometry, the [Ca\(^{2+}\)]\(_i\) response to thapsigargin was monitored in the absence or presence of specific inhibitors to classic PKC isoforms. Figure 1A shows a typical profile of the change in [Ca\(^{2+}\)]\(_i\), induced by thapsigargin and subsequent manipulation of bath calcium concentration ([Ca\(^{2+}\)]\(_o\)). Application of 1 μM thapsigargin in the presence of 1 mM [Ca\(^{2+}\)]\(_o\), evoked a rapid increase in [Ca\(^{2+}\)]\(_i\) to 180 nM, followed by a plateau phase of ~80 nM. On reduction of bath Ca\(^{2+}\) to <10 nM, the [Ca\(^{2+}\)]\(_i\) was lowered from the sustained stage to ~10 nM. Subsequent readmission of 1 mM Ca\(^{2+}\) to the bath induced an immediate increase in [Ca\(^{2+}\)]\(_i\) to 185 nM. This incremental change in [Ca\(^{2+}\)]\(_i\), in response to readmission of Ca\(^{2+}\), defined as Δ[Ca\(^{2+}\)], in the present study, is an indicator of Ca\(^{2+}\) entering the cell through SOC (28) and is equivalent to capacitative Ca\(^{2+}\) entry as depicted by Putney and McKay (41). Thus, in the following experiments using fura 2 ratiometry, we focused on the alteration in Δ[Ca\(^{2+}\)] induced by treatment of specific inhibitors of PKC isoforms.

Figure 1, B and C, shows the effects of inhibiting various PKC isoforms on the capacitative Ca\(^{2+}\) entry triggered by thapsigargin. Gö-6976, a selective inhibitor of both PKCa and βI in the range of 1 μM, significantly attenuated the thapsigargin-induced Ca\(^{2+}\) influx in response to readdition of Ca\(^{2+}\) to the bath (Δ[Ca\(^{2+}\)]: 113.9 ± 23.1 nM vs. 38.3 ± 14.9 nM, thapsigargin vs. thapsigargin plus 1 μM Gö-6976). However, such inhibition of SOC was not observed when treating with 500 nM LY-379196 (Δ[Ca\(^{2+}\)] = 117.0 ± 11.6 nM), an inhibitor of PKCb and βII, or with 100 μM HBDDE (Δ[Ca\(^{2+}\)] = 90.5 ± 19.7 nM), an inhibitor of PKCa and γ.

Effects of PKCa antisense on thapsigargin-induced capacitative Ca\(^{2+}\) entry. The near complete abolishment of Δ[Ca\(^{2+}\)] by Gö-6976 indicated that PKCa might be a specific mediator of capacitative Ca\(^{2+}\) entry. To further explore this notion, the thapsigargin-evoked rise in [Ca\(^{2+}\)]\(_i\) was examined in cells treated with PKCa antisense and scrambled oligonucleotides. As shown in Fig. 2, pretreatment with PKCa antisense (2.5 nM) for 1–2 days greatly depressed Δ[Ca\(^{2+}\)],. However, when MC were treated for 1–2 days with the same dose of scrambled nonsense oligonucleotides, Δ[Ca\(^{2+}\)] was not different from control (82.7 ± 16.8 nM vs. 113.9 ± 23.1 nM, scrambled sequence vs. control, P > 0.05; Fig. 2B).
**Patch-Clamp Experiments**

**Effects of various PKC isoform inhibitors on thapsigargin-induced activation of SOC in cell-attached patches.** The cell-attached configuration was employed to detect single-channel currents of SOC responding to thapsigargin in the presence and absence of specific inhibitors of PKC isoforms. Representative tracings of single channel currents are shown in Fig. 3A. Consistent with previous reports (26, 28), SOC have minimal spontaneous activity in basal conditions (NP_o 0.17). Depletion of internal Ca^{2+} stores by thapsigargin increased the NP_o to 0.26. The thapsigargin-induced response was ablated in the presence of Go-6976 (Fig. 3, A and B). However, neither LY-379196 nor HBDDE attenuated the currents activated by thapsigargin (Fig. 3, A and B). None of the three inhibitors significantly affected the basal activity of SOC (Fig. 3A).

**Effects of PKCα antisense on SOC in cell-attached patches.** The role of PKCα in the SOC signaling pathway was examined by pretreating MC with PKCα antisense or scrambled nonsense oligonucleotides before detecting the thapsigargin-evoked SOC responses. As shown in Fig. 4, in the presence of the scrambled nonsense sequence, application of thapsigargin still evoked a significant increase in open probability of SOC (by 98.3 ± 33.5%). However, in the group treated with PKCα antisense, thapsigargin evoked only a slight increase in NP_o (by 9.5 ± 3.3%). No significant difference in basal activity of SOC was detected when comparing the scrambled sequence and antisense-treated groups (NP_o 0.26 ± 0.09 vs. 0.25 ± 0.09).

**Effects of purified PKC isoforms on SOC in inside-out patches.** The inside-out configuration was employed to determine the effects of four classic purified isoforms of PKC on the single-channel SOC currents. In these experiments, as reported previously (26), a spontaneous decrease in SOC activity (rundown) was routinely observed after excision. When the channel activity obtained stability after excision, the specific PKC isoform was added to the bath. Because the classic PKCs require phospholipid and Ca^{2+} to be activated, 1 μM PMA, 100 μM Mg-ATP, and 1 mM Ca^{2+} were added to the solution with each PKC isoform. A previous study

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**Fig. 2.** Thapsigargin-induced Ca^{2+} entry in PKCα antisense or scrambled oligonucleotide-treated human mesangial cells (HMC; 2.5 nM for 24–48 h). A: representative tracings show [Ca^{2+}]_i responses to readdition of Ca^{2+} to the bath in a cell treated with PKCα antisense or scrambled nonsense. Thapsigargin was present in the bath throughout the experiment. Arrow indicates the time of readmission of Ca^{2+}. B: averaged data showing significant inhibition of Δ[Ca^{2+}], with PKCα antisense treatment. *Significant difference between antisense group and thapsigargin group.

**Fig. 3.** Effects of specific inhibitors of PKC isoforms on thapsigargin-induced activation of store-operated Ca^{2+} channels (SOC) in cell-attached patches. A: original tracings show the single-channel currents at basal condition, after application of inhibitors and inhibitors plus thapsigargin. Arrows indicate closed state of SOC. Downward deflections represent inward currents. B: summary data show effects of different inhibitors of PKC isoforms on open probability of SOC in the presence of thapsigargin. *Significant increases in NP_o of SOC. + Significant difference between Go-6976 and thapsigargin.
Fig. 4. Responses of SOC currents (cell-attached, \(-V_\text{o} = -80 \text{ mV}\)) to thapsigargin in HMC treated with PKC\(\alpha\) antisense or scrambled nonsense oligonucleotides (2.5 nM for 24 to 48 h). A: representative tracings showing thapsigargin-evoked activation of SOC in the PKC\(\alpha\) scrambled but not antisense treated cells. B: summary data. *Significant increase in open probability of SOC. †Significant difference compared with scrambled group.

Western blot analysis of expression of PKC\(\alpha\), \(\beta\), \(\beta\)II, and \(\gamma\) in cytosol and membrane fractions. The Ca\(^{2+}\) imaging and patch-lamp experiments suggested that PKC\(\alpha\) is a contributor to thapsigargin-induced activation of SOC. Results from inside-out patches suggested that SOC are activated by PKC\(\alpha\) and \(\beta\) cannot be attributed to PMA because this stimulatory effect was not observed for PKC\(\beta\)II and \(\gamma\) under the same conditions.

Expression of PKC\(\alpha\) under treatment with PKC\(\alpha\)-blocking peptide or PKC\(\alpha\) antisense. Analysis with fura 2 fluorescence ratiometry, patch clamping, and Western blotting consistently implicated PKC\(\alpha\) as a mediator in the activation of SOC by thapsigargin. To further investigate the notion that thapsigargin treatment triggers PKC\(\alpha\) translocation, PKC\(\alpha\) antibody was preincubated with specific PKC\(\alpha\) blocking peptide for 1 h before its addition to the nitrocellulose membrane, which had been transferred with PKC\(\alpha\) proteins. The immunoblotting bands, present in the cytosolic and membrane compartments of MC, were not detected after preabsorption of PKC\(\alpha\) antibody, indicating the specificity of the PKC\(\alpha\) protein detected in the present study.

In the fura 2 fluorescence and patch-clamp experiments, it was demonstrated that the PKC\(\alpha\) antisense treatment significantly attenuated the thapsigargin-
induced activation of SOC. To further illustrate that this depressed response was attributed to deficient PKCα, Western blotting was used to detect the expression of PKCα in samples pretreated with PKCα antisense and scrambled nonsense. As shown in Fig. 7, specific immunoblotting bands for PKCα were detected in both cytosolic and membrane fractions from cells treated with scrambled oligonucleotides. However, the bands in both fractions were reduced in antisense treated samples. As shown, no difference in the expressions of PKCβI, PKCβII, or PKCγ was observed between scrambled and PKCα antisense-treated cells. Therefore, the antisense-induced depression is selective for PKCα.

**DISCUSSION**

Depending on the specifically tested cells and the experimental conditions, variable results have been reported on the modulation of SOC by PKC (1, 2, 7, 39, 46, 51). The differential tissue distribution, intracellular localization, and cellular functions of different isoforms of PKC might also contribute to these discrepancies. Using fura 2 fluorescence measurements combined with patch clamping, we previously demonstrated that PKC activates SOC through a phosphorylation mechanism (26). The previous findings are extended by the current study, which detects specific isoforms of PKC involved in this signaling pathway. The data of the present study showed the following: 1) Gö-6976, a PKCα and βI inhibitor, significantly attenuated thapsigargin-induced capacitative Ca2+ entry measured by fura 2 fluorescence and single-channel analysis; 2) purified PKCα and βI, but not PKCβII and γ, reactivated SOC from postexcision rundown; 3) specific PKCα antisense depressed Ca2+ influx stimulated by thapsigargin; and 4) thapsigargin-induced depletion of internal Ca2+ stores triggered translocation of PKCα and γ, but not βI and βII, from the cytosolic to membrane cellular fractions. These results indicate that PKCα plays an important role in regulating activity of SOC.

**Influences of selective inhibitors of various PKC isoforms.** Within a restricted concentration range, a selective inhibitor of a specific PKC isoform might still affect another isoform to some extent. This problem must be considered when interpreting results utilizing pharmacological tools. In the present study, 1 μM Gö-6976, an inhibitor of both PKCα and βI, significantly depressed the thapsigargin-induced capacitative Ca2+ entry assessed by Ca2+ imaging (Fig. 1). This inhibition was corroborated with electrophysiological methods (Fig. 3), implying that either PKCα or βI (or both) mediate the thapsigargin-evoked activation of SOC. Interestingly, inhibiting PKCα and γ by HBDDE or PKCβI and βII by LY-379196 failed to suppress the thapsigargin-induced responses. These results could be explained by opposing effects of PKCβII or γ with PKCβI and α on SOC, respectively. Thus the stimulatory effects from PKCα or βI were compromised by the inhibitory effects from PKCγ or βII. Indeed, opposite effects of different isoforms of PKC on the same cellular events have been reported by many groups of investi-
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The data from inside-out patches further suggested that PKCa and βI are able to activate SOC directly (Fig. 5). However, the possible inhibitory effects of PKCβII and γ could not be detected with the inside-out configuration because the channel activity had already been minimized after excision.

Identification PKCa as a mediator for thapsigargin-induced activation of SOC. When activated, PKC normally translocates to its target site, which, in the case of SOC, is located in the plasma membrane. The results of Western blotting revealed that only PKCa and γ translocated from cytosol to membrane in response to thapsigargin (Fig. 6). However, this trafficking could not be observed for PKCβI and βII. These experiments suggest that PKCa and γ are part of the signaling pathway involving the activation of SOC after depleting internal Ca²⁺ stores.

Two apparent paradoxes remain when comparing the results from Western blot analysis with those from the fura 2 fluorescence and patch-clamp experiments. The first paradox is that PKCβI significantly reversed SOC run down after excision but was not translocated from cytosol to membrane in thapsigargin-treated MC. Thus, although PKCβI has the capacity to activate SOC, it may not contribute to the activation of SOC when stores are depleted. The second paradox is that PKCγ translocated from cytosol to membrane in response to thapsigargin in the Western blot experiments but did not reactivate SOC when applied directly to inside-out patches. One explanation is that membrane components other than SOC are substrates for PKCγ. Alternatively, PKCγ could inhibit SOC after translocating to the plasma membrane. An inhibitory effect would not be apparent in inside-out patches because SOC runs down nearly completely after excision. It is also possible that a thapsigargin-evoked increase in cytosolic Ca²⁺ caused PKCγ to move to the plasma membrane. However, the translocation of PKCγ was not examined in the presence of BAPTA-AM, the intracellular Ca²⁺ buffer, in the current study.

The experiments utilizing PKCa antisense provided additional support for the notion that PKCa is a key component mediating thapsigargin-evoked activation of SOC in MC. Treating MC with antisense oligonucleotides specific for PKCa attenuated thapsigargin-induced capacitative Ca²⁺ influx as measured by fura 2 ratiometry and completely inhibited thapsigargin-evoked activation of SOC determined by the cell attached patch-clamp method.

The PKC superfamily is composed of twelve members, which are further subdivided into three groups: conventional, novel, and atypical (6, 35, 36). Because specific inhibitors are presently available only to conventional PKCs, the possibility that one or more conventional PKCs are involved in the intracellular pathway for activating SOC has been examined in the current study. Even though these data suggest that PKCa participates in thapsigargin-induced activation of SOC, the results do not eliminate the possible involvement of other isoforms of PKC or other mechanisms of regulating the channel activity.

It was interesting that PKCa antisense and Gö-6976 completely abolished the thapsigargin-evoked activation of SOC determined by the patch-clamp technique but failed to completely abolish the thapsigargin-induced Ca²⁺ entry determined by fura 2 measurements. There are two possible explanations for these apparent contradictory results. First, fura 2 measures global intracellular Ca²⁺ concentration. It is possible that PKCa also stimulates the extrusion of Ca²⁺ via Na⁺/Ca²⁺ exchange after it enters the cell via SOC channels. In this case, an inhibitor of PKCa would completely prevent the thapsigargin-evoked increase in [Ca²⁺], but it would not completely block a rise in [Ca²⁺]. Second, in the fura 2 experiments, the residual Gö-6976-insensitive Ca²⁺ entry could have been through other Ca²⁺ permeable ion channels, such as a nonselective cation or the voltage-gated Ca²⁺ channel, previously described in these cells (13).

Recently, one group of investigators reported that activation of phospholipase C (PLC) activated expressed TRP3 channels in DT40 chicken B lymphocytes in which all three inositol 1,4,5-trisphosphate receptors (IP3R) were deleted (49). Activation of TRP3, a reportedly strong candidate for the store-operated channel (48, 49), was blocked by the PLC inhibitor, U-73122. Importantly, the diacylglycerol (DAG) analog 1-oleoyl-2-acetyl-sn-glycerol also activated TRP3 channels independently of IP3R. Because DAG is a crucial cofactor for conventional and novel isoforms of PKC, the results from that study support the hypothesis that one or more isoforms of PKC participate in activating SOC after store depletion. However, it should be noted that an earlier study (15) found that DAG directly activated human TRP3 and TRP6 through a PKC-independent mechanism.

It is not understood how PKCa is activated after depletion of internal Ca²⁺ stores. Although cytosolic Ca²⁺ concentration is elevated on depleting Ca²⁺ stores, it is probably not a primary mechanism for activating SOC. Previous studies from this laboratory and others have shown that SOC is activated upon store depletion despite the clamping of Ca²⁺ with intracellular buffers (16, 26). Supporting this notion are the results of one group that recently investigated the regulation of PKCa by temporal and spatial changes in [Ca²⁺]. Maasch et al. (29) demonstrated that the thapsigargin-induced elevation of cytosolic Ca²⁺ targeted PKCa to distinct intracellular compartments but not the plasma membrane. It is possible, however, that an unknown PKC-stimulating phospholipid is generated on depleting internal Ca²⁺ stores. It is also possible that, on depletion of ER Ca²⁺, the cytoskeleton rearranges and activates PKC. It has been shown previously that disruption of the actin cytoskeleton activates PKCa in mesenchymal cells (25). Moreover, it was reported that calponin, a cytoskeletal protein, may serve to regulate PKC by facilitating its phosphorylation (24). A recent study revealed that a functional and integral actin microfilament network is essential for translocation of PKCa from the cytosol to the plasma membrane (47).
Another question relates to how phosphorylation by PKCa activates SOC. PKCa might phosphorylate SOC via a direct enzyme-substrate reaction. However, it is more likely that the effect of PKCa on SOC is mediated by a specific scaffolding protein. It has been proposed that many PKC-evoked cellular responses require particular receptor proteins that anchor PKC to its specific targets (8, 20, 21). Interestingly, in Drosophila, an eye-specific protein kinase C (InaC) forms a supermolecular complex with TRP and two other proteins, norpA-encoded phospholipase C and InaD protein (17). InaD is a putative substrate of InaC and might serve as an anchoring protein for InaC.

In conclusion, the present study strongly suggests that PKCa participates in the intracellular pathway mediating activation of SOC by depletion of internal Ca\(^{2+}\) stores in MC.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-49561 (to S. C. Sansom), a fellowship grant from American Heart Association (Heartland Affiliate) (to R. Ma), and National Heart, Lung, and Blood Institute Research Training Grant 1T32-HL-07888 (to P. Kudlacek).

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