Osmomechanical stress selectively regulates translocation of protein kinase C isoforms

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Abstract Osmomechanical stress, resulting in cell swelling and activation/regulation of numerous cellular processes, may play a critical role in cell signaling by selectively regulating translocation of protein kinase C (PKC) isoforms from cytosol to membrane compartments. Western blotting of renal epithelial cell fractions demonstrated the expression of five PKC isoforms. Three of these isoforms (PKCα, PKCε, PKCζ) translocated to the membrane fraction upon exposure of cells to osmomechanical stress (hypotonic medium). Immunohistochemical staining of cells using isoform-specific antibodies further demonstrated translocation of the phorbol ester-sensitive isoforms, PKCα and PKCε, to both the plasma membrane and perinuclear sites, reflecting potential initial steps in regulation of specific effector pathways. Indeed, selective inhibition of PKCs indicates a potential role for PKCα in modulating a calcium influx channel. It is concluded that osmomechanical stress induces selective translocation of specific PKC isoforms, demonstrating a key role of osmomechanical stress in selectively regulating PKC-dependent signaling pathways.

Key words: Mechanical stress; Swelling; Hypotonic; Protein kinase C; Translocation; Epithelium

1. Introduction

The protein kinase C (PKC) isozymes represent a large family of protein kinases that play central roles in signal transduction pathways associated with neurotransmitters, peptide hormones, and growth factors [1,2]. The coupling of PKCs to initiating signals and transduction pathways is notoriously diverse leading to a broad range of functions regulated/modulated by PKCs, including cell proliferation and migration [1,2], vasoconstriction [3], secretion [4], cell shape and volume [5,6], and solute transport [2,7,8]. Part of this diversity is due to the diverse properties of the PKC isoforms themselves. There are 11 known mammalian isoforms of PKC that are typically divided into three groups according to the structural/functional domains of the regulatory subunit [1,2]: (1) the conventional PKCs, cPKCs (α, βI, βII and γ isoforms); (2) the novel PKCs, nPKCs (ε, δ, η, and θ isoforms); and (3) the atypical PKCs, aPKCs (ζ, λ, and ι isoforms). The cPKCs are activated by Ca and diacylglycerol (DAG)/phorbol esters, the nPKCs by DAG/phorbol esters only and not by Ca, and the aPKCs are not sensitive to either Ca or DAG/phorbol esters. A related kinase that was initially considered part of the PKCs, PKCμ, is often considered to be a distantly related isozyme that may belong to a separate family (PKD). Its mode of regulation is poorly understood.

In recent years it has become apparent that the role of PKCs in signal transduction may extend beyond the simple ligand/receptor-coupled transduction pathways. Indeed, it has been shown that mechanical stimuli, such as membrane stretch or shear stress, are often linked in diverse ways through various transduction molecules and pathways within most cells [5,9,10]. Most recently it has been demonstrated that osmomechanical stress, such as hypotonic-induced cell swelling, may itself be a major stimulus regulating numerous processes in the cell including proliferation, growth, secretion, and cell volume/shape [5,11,12]. In some cases it has been demonstrated that PKCs may be central for regulating/modulating the osmomechanically induced processes. It has been shown that PKCs may underlie numerous processes modulated by osmomechanical stress including osmomechanical modulation of Ca channels [13,14], anion channels [15,16], mitogen-activated protein kinase cascades [5,17], and hormone secretion [18] to name a few. Hence, it is becoming apparent that osmomechanical transduction pathways are a central component of most cells and that the control of the associated transduction pathways may, in numerous cases, be modulated by PKC isozymes.

The purpose of the current study was to determine if osmomechanical stress, leading to cell swelling, differentially controls the translocation and distribution of specific PKC isoforms as a potential step in PKC activation. Renal epithelial cells were utilized as it has been shown that osmotic swelling of these cells leads to activation of phospholipase C to generate DAG [13,19] and, in turn, PKC-dependent processes, including a swelling-activated calcium channel [14,20]. The function of specific PKC isoforms in these cells is currently unknown.

2. Materials and methods

2.1. Cell culture

Rabbit proximal tubule cells (PT cells) were isolated according to the methods of [21], as we have previously done [13,20]. Cells were grown to confluence in 100×20-mm dishes (Corning Glass Works, Corning, NY, USA) or on coverslips (see below) in Dulbecco's modified Eagle's medium/Ham's F-12 nutrient mixture (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Life Technologies, Gaithersburg, MD, USA), 1.06 g/l $NaHCO_3$, 100 000 U/l pen-

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icillin G sodium (Gibco BRL, Gaithersburg, MD, USA), 100 mg/l streptomycin sulfate (Gibco BRL), 10 mg/l gentamicin (Sigma), 50 μM hydrocortisone, 10 mg/l transferrin, and 5 mg/l insulin. The cells were incubated in a humidified atmosphere of air and 5% CO_2 at 37°C. Cell passages 4–7 were used in the present study.

2.2. Experimental protocol

The effects of osmomechanical stress due to swelling on PKC isoform distribution among different cellular compartments were determined. Three treatment groups of PT cells were used. The first treatment group was treated only with isotonic bathing medium and was considered as control (ISO, 310 mOsm/kg water). The ISO solution contained (in mM): 110 NaCl, 4.2 KCl, 0.4 Na_2HPO_4, 0.5 NaH_2PO_4, 1.0 $CaCl_2$, 0.3 $MgSO_4$, 20 HEPES, and 90 mannitol, pH 7.4. The second group was treated with hypotonic bathing medium (HYPO, 220 mOsm/kg water) to induce cell swelling. The HYPO solution was identical to the ISO solution except that the mannitol was removed to reduce the osmolality to 220 mOsm/kg. The third group was treated with phorbol 12-myristate 13-acetate (PMA) (Sigma) in isotonic bathing medium to directly activate PKC (PMA group). The cells were then harvested and fractionated as described below.

2.3. Cell fraction preparation

Confluent PT cells were washed twice with room temperature phosphate-buffered saline (PBS) and incubated with isotonic solution – Hanks' balanced saline solution (HBSS) adjusted with mannitol to 307 mOsm – for 45 min at room temperature. HBSS contains 0.1 M NaCl, 4.2 mM KCl, 0.4 mM Na_2HPO_4, 0.5 mM NaH_2PO_4, 0.3 mM $MgCl_2$, 0.4 mM $MgSO_4$, and 20 mM HEPES, pH to 7.4. The cells were then exposed to the various treatments: ISO, HYPO (5 min) or PMA (1 μM, 10 min). After treatment, the cells were scraped with ice-cold homogenization buffer (20 mM Tris–HCl, 0.5 mM EDTA, 0.5 mM EGTA, 2 mM dithiothreitol, 300 mM sucrose, 10 μg/ml pepstatin, 1 μg/ml leupeptin, and 25 μg/ml aprotinin, pH to 7.4). The cell suspension was centrifuged at $500\times g$ for 5 min at 4°C, the pellet was resuspended in the homogenization buffer and minced via 15–16 strokes in the prechilled homogenizer in the ice bucket. The total cell fraction homogenate was centrifuged again at $145\,000\times g$ for 30 min at 4°C. The supernatant was used as the cytosolic fraction. The pellet, the microsomal fraction, was resuspended and incubated in the homogenization buffer containing 1% Triton X-100 under constant shaking for 30 min at 4°C. The Triton X-100 extract underwent centrifugation at $145\,000\times g$ for 30 min at 4°C. The supernatant was used as the microsomal membrane fraction. The pellet was discarded. Finally, total amounts of protein in the microsomal fraction and cytosolic fraction of PT cells were quantified using the BCA assay reagent kit (Pierce, Rockford, IL, USA) according to the manufacturer's instruction.

2.4. Western blotting analysis of PKC isoform

Equal amounts of microsomal and cytosolic proteins (5 μg or 10 μg) from each sample were separated by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto activated polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA). After transfer, the PVDF membranes were blocked in 5% non-fat milk in PBS at 4°C overnight. Primary antibodies for each PKC isoform (PKCα, βI, βII, γ, ε, δ, η, θ, ζ, λ, and ι) and PKCμ (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:1000 in 2.5% non-fat milk in PBS–Tween were used for immunoblotting. The membranes were probed with primary antibody for 1 h with mild shaking at room temperature followed by three washes with PBS–Tween for about 30 min. The blots were incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (1:5000) (Amersham Life Science, Arlington Heights, IL, USA), as appropriate, as the secondary antibody for 1 h at room temperature. After extensive washes with PBS–Tween for about 30 min, the bound antibodies was visualized on ECL Hyperfilm (Amersham), using an ECL detection reagent chemiluminescence system (Amersham). The quantification of the appropriate PKC bands was done by densitometry reading on Hewlett Packard Version 2.2 ScanJet 3c/T. The signals were then analyzed by Sigma Gel (Jandel Scientific, San Rafael, CA, USA) and the densities expressed in arbitrary units.

2.5. Immunofluorescent assessment of PKCα and PKCε translocation

Only two DAG-sensitive PKC isoforms, PKCα and PKCε, were shown to be sensitive to osmomechanical stress based on cell fractionation and Western blot studies (see Section 3). To assess further the translocation of these two isoforms, additional immunofluorescent microscopy studies were done to define the distributions of the isoforms. PT cells were grown to confluence in the four-well chamber slides. The cells were washed twice with isotonic room temperature PBS before undergoing the different treatments. The cells were divided into the three treatment groups: the first two wells were bathed in ISO, the third well was bathed in HYPO, whereas the fourth well was bathed in ISO with 1 μM PMA. Cells were incubated in each test solution for 5 min, typically (3-min and 15-min treatments were used in a few studies), to test if there was any change in PKC localization in the subcellular level over this time period. At the end of the treatment the solutions were immediately removed from the chamber slides and 4% paraformaldehyde was added for 15 min at room temperature to fix the cells. After fixation the cells were extensively washed with PBS twice for 30 min at room temperature. The cells were then permeabilized with a 50:50 mixture of methanol and acetone for 60 s followed by PBS washes, twice, over 15 min. Next the cells were blocked with 1:50 normal goat serum (NGS) in PBS for 1 h at room temperature, and subsequently incubated with the primary antibody in a NGS and PBS mixture (1:50) overnight at 4°C. The same polyclonal rabbit anti-PKCα/ε antiserum as in the Western blots was used in the current study. The next day cells were washed with PBS–0.1% bovine serum albumin (BSA), twice, over 60 min, with rocking, at room temperature. Oregon green-conjugated goat anti-rabbit antibody was then applied at a concentration of 1:300 in PBS–0.1% BSA to the cells for 1 h at room temperature. All procedures were protected from light from this point on. Following two washes with PBS–0.1% BSA and a 5-min wash with PBS at room temperature, the cells were counterstained with Hoechst dye (1:200 in PBS) for 60 s, and again washed with PBS and distilled water for 10 min each. Finally, coverslips were placed over the cells and the cells photographed using a Nikon Optiphot2 microscope equipped for epifluorescence.

2.6. Measurement of intracellular calcium levels

Intracellular calcium levels were measured with the calcium-sensitive fluorescent Fura 2 probe using standard techniques as done before [13,14]. Cells on coverslips were loaded with Fura 2/AM for 45 min, washed, and mounted on the microscope stage of a fluorescent imaging workstation (Intracellular Imaging) at room temperature. Cells were repetitively excited at 340 nm and 380 nm and the emission at 511 nm ratioed to provide a measure of intracellular calcium levels as outlined previously [13,14]. In a typical experiments, cells were initially bathed with the ISO solution (see above) for a control period (10–20 min) and then subjected to test conditions (10–20 min) by either addition of HYPO medium, to induce osmotic swelling, or addition of the DAG analogue, 1-oleoyl-2-acetyl-*sn*-glycerol (OAG, 2 μM), to activate PKCs. Both test conditions have been shown to activate Ca influx and, hence, rapidly increase intracellular Ca levels [13,14]. Intracellular Ca measurements were made continuously throughout the control and test periods.

2.7. Statistical analysis

Statistical analysis was performed with the SigmaStat program (Jandel Scientific), using one-way ANOVA followed by the Student–Newman–Keuls *t*-test to determine significance. The data are presented as means ± S.E.M. $P < 0.05$ was considered significant.

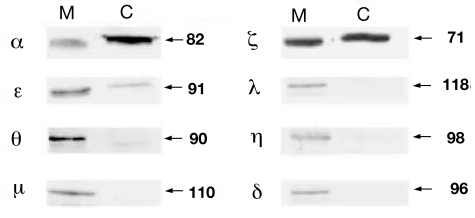

Fig. 1. Summary of PKC isoform Western blot analysis. Immunoblots of eight PKC isoforms identified in rabbit PT epithelial cells showing distribution patterns for membrane (M) and cytosolic (C) fractions. Each isoform is indicated along with approximate molecular weight. The most abundant isoforms were PKCα, ε, μ, θ and ζ, while PKCδ, η, and λ were detectable at low levels. Four other isoforms, PKCβI, βII, γ, and ι, were typically not detected.

Table 1
Summary of PKC isoform Western blot analysis in PT cells[a]

PKC subfamily	Isoform	Identified
Conventional	α	++
	βI	−
	βII	−
	γ	−
Novel	δ	+
	ε	++
	η	+
	θ	++
Atypical	ζ	++
	λ	+
	ι	−
PKCμ (PKD)	μ	++

++: Isoform detected in both membrane and cytosolic fractions; +: isoform detected in membrane fraction only of at least one blot; −: isoform not detectable in either membrane or cytosolic fractions.
[a]Two to nine blots were analyzed for each isoform.

3. Results and discussion

3.1. Distribution of PKC isoforms in PT cells

PKC isoforms were found to be widely expressed in PT cells. Based on Western blots of cell fractions, the abundance and distribution of all 11 PKC isoforms and PKCμ were evaluated in the PT epithelial cells. Five isoforms were abundantly expressed as shown in the summary data in Fig. 1 and Table 1. PKC isoforms α, ε, θ, ζ and μ were readily detected in both cytosolic and plasma membrane fractions of control (ISO) cells. In contrast, PKCβI, βII, γ, and ι isoforms were not detectable in either fraction demonstrating little if any expression of these isoforms in these cells under the current conditions. Similarly, PKCλ, δ, and η isoforms were normally not detectable in membrane and cytosolic fractions, although low-density bands were occasionally evident in membrane fractions only (see Fig. 1). The expression of these latter isoforms was not sufficient to assess HYPO-induced translocation from the cytosol to the plasma membrane with current techniques and, hence, these isoforms were not studied further.

Finally, of the five isoforms abundantly expressed in the PT cells, the specificity of the primary antibodies to the isoforms was assessed by competitive binding of a blocking peptide to the antibody. After detecting appropriate bands on Western blots with the primary antibody, the PVDF membranes were stripped and the primary antibody reapplied to the same PVDF membranes, but in the presence of the appropriate blocking peptide. In all cases the appearance of the isoform bands was significantly diminished as demonstrated by the example for PKCα in Fig. 2A (inset).

3.2. Effect of osmomechanical stress on PKC isoform translocation

The effect of osmomechanical stress on PKC isoform distribution between cytosol and cell membrane was determined by exposing the cells to HYPO. After treatment of cells with HYPO for 5 min, the cells were fractionated and isoform

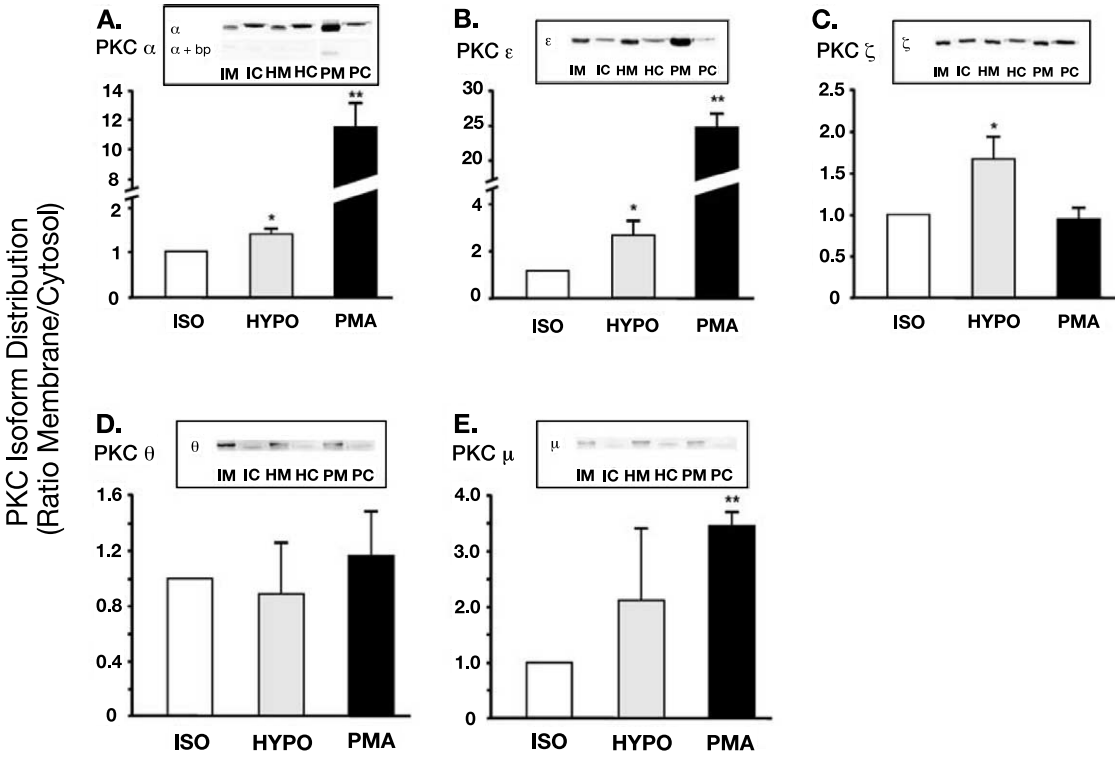

Fig. 2. Immunoblots of PKC isoforms from cells treated with HYPO or PMA. Confluent PT cells were treated with isotonic solution (ISO), hypotonic solution (HYPO, 220 mOsm/l) or PMA (1 μM) in ISO for 5, 5, and 10 min, respectively, and membrane and cytosolic fractions collected and immunoblotted. Fractions were probed with specific anti-PKC antibodies: (A) PKCα ($n=4$), (B) PKCε ($n=6$), (C) PKCζ ($n=5$), (D) PKCθ ($n=4$), (E) PKCμ ($n=5$). Only bands from the same gel were compared. All values shown are arbitrary densitometric units expressed as ratio fold of membrane vs. cytosolic fractions. Gel shown in inset is a representative Western blot of respective PKC isoform with the following loading sequence: IM: ISO membrane fraction. IC: ISO cytosolic fraction. HM: HYPO membrane fraction. HC: HYPO cytosolic fraction. PM: PMA membrane fraction. PC: PMA cytosolic fraction. 'α+bp' (A) is the PKCα antibody with a specific blocking peptide (bp) for PKCα. *$P<0.05$ HYPO compared to ISO; **$P<0.05$ PMA compared to ISO.

distribution assessed via Western blotting. As shown by the data in Fig. 2, two DAG-sensitive isoforms were shown to be sensitive to HYPO treatment. Both PKCα (Fig. 2A) and PKCε (Fig. 2B) displayed a translocation to the plasma membrane following HYPO as compared to cells treated only with ISO, demonstrating osmomechanical control of the distribution of these two isoforms. The increase for PKCα was modest, increasing by 40 ± 10%, while that for PKCε was more substantial, increasing by 253 ± 68%. Since these two isoforms belong to the DAG-sensitive groups, cPKC and nPKC, respectively, we tested to determine if PMA similarly would activate the translocation of these two isoforms. Indeed, as expected, PMA treatment (1 μM, 10 min) resulted in a major shift of these isoforms from the cytosol to the membrane fraction, increasing by over 11-fold (Fig. 2A) and 22-fold (Fig. 2B), respectively.

The aPKC, PKCζ, was also found to be sensitive to osmomechanical stress. As shown in Fig. 2C, a significant membrane translocation of PKCζ occurred in the HYPO treatment group (166 ± 28% increase) compared with the control ISO group. Treatment with PMA had little effect, as expected, for a DAG-insensitive isoform. It was somewhat unexpected to observe a sensitivity of PKCζ to HYPO in these cells as it is often considered that HYPO-induced processes in these cells are regulated via DAG-sensitive PKCs [13]. The significance of this response is currently not known.

Some PKCs were found to be insensitive to osmomechanical stress. As shown by the example in Fig. 2D, the distribution of PKCθ was not sensitive to HYPO treatment, nor to PMA treatment. Similarly, the distribution of PKCμ (Fig. 2E) or PKD was not altered by HYPO treatment although a small stimulation by PMA treatment was evident. Hence, not all PKC isoforms are regulated by osmomechanically controlled signaling pathways.

The present study demonstrates, for the first time, that osmotic cell swelling alone can lead to selective translocation and distribution of specific PKC isoforms to cell membrane domains. The three PKC isoforms observed to partition to the cell membranes, namely PKCα, PKCε, and PKCζ, represent isoforms from each of the three families of PKC, cPKC, nPKC, and aPKC, respectively. Hence, translocation and distribution of the isoforms is not a property of the type of PKC regulatory domain per se, but may reflect activation of multiple regulatory pathways important for each of the isoform subfamily members.

3.3. *Immunofluorescent assessment of PKCα and PKCε distribution*

It has been shown previously in PT cells that osmomechanical stress leads to activation of plasma membrane Ca channels through DAG-sensitive PKC pathways [13,14]. Of the three osmomechanically sensitive PKCs identified in the present study, two are DAG-sensitive, PKCα and PKCε. To assess the potential cellular sites of activation of these two isoforms and, hence, the types of processes that may be regulated by these isoforms, the subcellular localization of these two isoforms in the cultured PT cell was determined by immunofluorescent microscopy techniques.

Under control ISO conditions, PKCα appeared to be diffusely distributed throughout the cells (Fig. 3B) with little or no localization to the plasma membrane. Upon exposure to HYPO treatment (Fig. 3C) for 5 min, a redistribution of the isoform was apparent with modest translocation to both the plasma membrane (small arrows) and the perinuclear area (large arrowheads). The dominant redistribution, however, was to the perinuclear area, likely reflecting localization to the endoplasmic reticulum/Golgi/nuclear membranes. Hence, HYPO-induced translocation of PKCα to the plasma membrane, while evident, appears to reflect only a modest component of the isoform translocation following HYPO exposure. In contrast, when cells were exposed to PMA treatment, a marked redistribution of PKCα to both the plasma membrane and perinuclear area was apparent (Fig. 3D). To determine if the redistribution patterns were altered over several minutes of exposure to each treatment, the above 5-min treatment conditions were compared to treatment periods of 3 and 15 min. No obvious alterations in distribution were apparent during these time periods (data not shown).

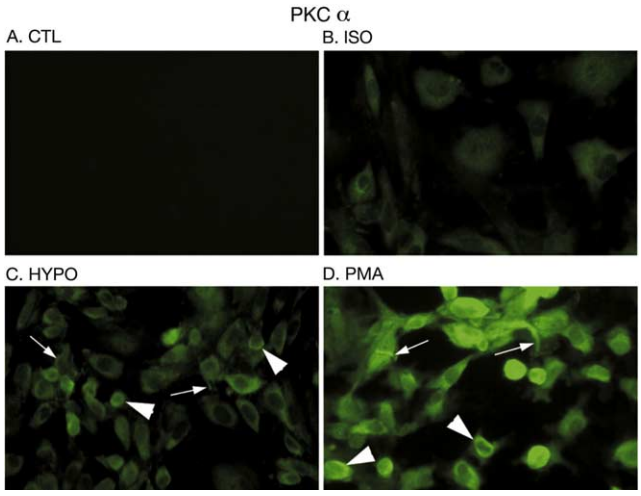

Fig. 3. Immunofluorescent images of PKCα distribution. The cells were grown to confluence in four-well chamber slides followed by treatments with ISO, HYPO, or PMA solutions, respectively, for 5 min. Cells were then immunostained for PKCα using the anti-PKCα antibody and photographed using a Nikon Optiphot2 microscope equipped for epifluorescence (magnification 20×) as described in Section 2. Small arrows indicate plasma membrane localization and large arrowheads indicate perinuclear localization. CTL: control without primary antibody; ISO: isotonic treatment; HYPO: hypotonic treatment; PMA: PMA treatment.

The distribution of PKCε was also found to be sensitive to HYPO treatment. Under control ISO conditions the isoform was diffusely distributed throughout the cell with no apparent plasma membrane localization (Fig. 4B). Upon exposure to HYPO treatment for 5 min (Fig. 4C), PKCε demonstrated a significant translocation to both the plasma membrane (small arrows) and the perinuclear area (large arrowheads). In addition, the isoform distribution within the cytoplasm also displayed a weak reticular pattern consistent with modest redistribution to cytoskeletal structures. HYPO treatment would appear to lead to redistribution of the isoform to several key sites within the cell, likely reflecting a regulatory role in multiple cellular processes. Finally, with PMA treatment, PKCε redistribution followed the same pattern as that observed with HYPO treatment, but with a more extensive redistribution (Fig. 4D). Similar to that observed with PKCα, treatment of cells for 3 or 15 min did not obviously alter the distribution patterns of PKCε (data not shown).

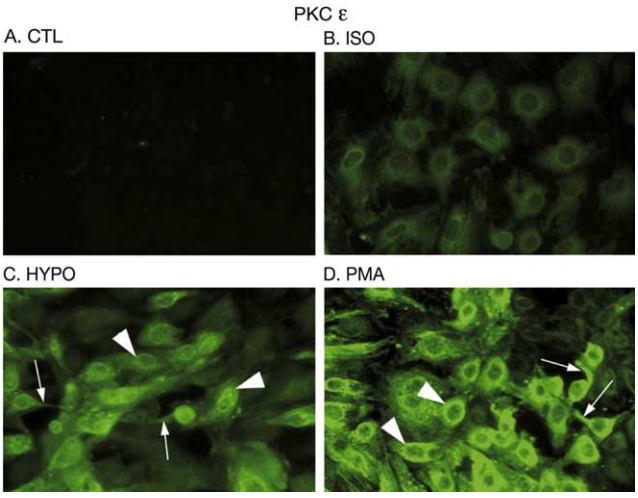

Fig. 4. Immunofluorescent images of PKCε distribution. The cells were grown to confluence in four-well chamber slides followed by treatments with ISO, HYPO, or PMA solutions, respectively, for 5 min. Cells were then immunostained for PKCε using the anti-PKCε antibody and photographed using a Nikon Optiphot2 microscope equipped for epifluorescence (magnification 20×) as described in Section 2. Small arrows indicate plasma membrane localization and large arrowheads indicate perinuclear localization. CTL: control without primary antibody; ISO: isotonic treatment; HYPO: hypotonic treatment; PMA: PMA treatment.

Regulation of PKC isoform translocation and activation is thought to be a relatively specific process that is typically localized to specific subcompartments within the cell thereby resulting in selective activation of pathways within the subcompartments. This subcompartment specificity may result from regulation of protein trafficking to distinct sites, as previously demonstrated [22]. Alternatively, the specificity may be controlled by binding proteins or receptors for PKCs, the receptor-activated C kinases (RACKs) [23], as the location of the RACKs can provide the compartmental specificity. The role of RACKs in regulating PKC activation in PT cells is not known. However, it was shown for the first time in the present study that PKCα and PKCε are translocated to specific sites within the cells following HYPO-induced or PMA-induced activation. Both isoforms appear to distribute to sites within the plasma membrane and to perinuclear regions, common sites of translocation for some PKC isoforms [24–26], and likely leads to phosphorylation of signaling components at these localized sites which, in turn, regulate downstream effectors such as plasma membrane ion channels or signaling pathways modulating gene expression, respectively.

3.4. PKCα regulation of calcium influx

As heretofore noted, osmomechanical stress activates a Ca influx pathway through a PKC-dependent mechanism in the renal cells [13,14,20]. Since OAG, a DAG analogue, and PMA can also activate this pathway [14], it is likely that the DAG-sensitive PKCs, such as PKCα or PKCε, regulate calcium influx. As an initial assessment of PKCα or PKCε specificity for this pathway, the effects of selective PKC inhibitors on Ca influx were determined using the change in intracellular Ca levels following activation by HYPO or OAG (2 μM) as an index of Ca influx as done before [13,14]. (That is, stimulation of Ca influx through the native Ca channels by HYPO or OAG treatment would lead to a rapid rise in intracellular Ca levels while inhibition of the Ca influx channels would lead to depressed intracellular Ca levels.) Intracellular calcium levels were measured via the Fura 2 fluorescence method. Addition of Go 6976 (Calbiochem) (200 nM), a relatively specific blocker of PKCα (over PKCε), significantly reduced the normal OAG-induced peak change in calcium from 252 ± 29 nM ($n = 6$) to 115 ± 25 nM ($n = 6$) after Go addition ($P < 0.05$), and the HYPO-induced change from 99 ± 11 nM ($n = 7$) to 64 ± 6 nM ($n = 7$) after Go addition ($P < 0.05$). Hence, Go 6976 treatment reduced the stimulated Ca influx. In contrast, addition of Ro-31-8425 (Calbiochem), a more general blocker of PKCε and similar PKCs, did not further reduce Ca influx, i.e. it did not further reduce intracellular Ca levels (data not shown). Hence, these initial studies point to a specific role for PKCα in regulating HYPO-induced Ca influx in these cells.

In summary, it has been shown that osmomechanical stress is an important 'signaling' component controlling translocation of specific PKC isoforms. Specific PKC isoforms from all three subfamilies of PKC, namely cPKC, nPKC, and aPKC, were shown to be sensitive to swelling-induced states. The distribution patterns of translocation included plasma membranes, perinuclear membranes, and cytoskeletal structures, demonstrating a broad array of processes likely controlled by these isoforms during osmomechanical stress. While some of the processes regulated by these isoforms is known, such as swelling-activated calcium channels and the associated calcium signaling, other processes controlled by these isoforms are yet to be defined.

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References

[1] Nishizuka, Y. (1995) FASEB J. 9, 484–496.
[2] Webb, B.L., Hirst, S.J. and Giembycz, M.A. (2000) Br. J. Pharmacol. 130, 1433–1452.
[3] Abdel-Latif, A.A. (2001) Exp. Biol. Med. 226, 153–163.
[4] Gilon, P. and Henquin, J.C. (2001) Endocr. Rev. 22, 565–604.
[5] Kim, R.D., Stein, G.S. and Chari, R.S. (2001) J. Cell Biochem. 83, 56–69.
[6] Ottaviani, E., Franchini, A. and Kletsas, D. (2001) Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 129, 295–306.
[7] Farese, R.V. (2002) Am. J. Physiol. Endocrinol. Metab. 283, E1–E11.
[8] Mackay, K. and Mochly-Rosen, D. (2001) J. Mol. Cell. Cardiol. 33, 1301–1307.
[9] Ali, M.H. and Schumacker, P.T. (2002) Crit. Care Med. 30 (5 Suppl.), S198–S206.
[10] Li, C. and Xu, Q. (2000) Cell Signal. 12, 435–445.
[11] Haussinger, D., Graf, D. and Weiergraber, O.H. (2001) J. Nutr. 131 (9 Suppl.), 2509S–2514S.
[12] McCarty, N.A. and O'Neil, R.G. (1992) Physiol. Rev. 72, 1037–1061.
[13] O'Neil, R.G. and Leng, L. (1997) Am. J. Physiol. 273, F120–F128.
[14] Zhang, M.I.N. and O'Neil, R.G. (2001) Cell Calcium 29, 263–275.
[15] Vanoye, C.G., Castro, A.F., Pourcher, T., Reuss, L. and Altenberg, G.A. (1999) Am. J. Physiol. 276, C370–C378.
[16] Vieira, L.L., Lafuente, E., Blum, J. and Cabantchik, Z.I. (1997) Mol. Biochem. Parasitol. 90, 449–461.
[17] Shen, M.-R., Chou, C.-Y., Browing, J.A., Wilkins, R.J. and Ellory, J.C. (2001) J. Physiol. 537, 347–362.
[18] Strbak, V. and Greer, M.A. (2000) Cell. Physiol. Biochem. 10, 393–402.
[19] Suzuki, M., Kawahara, K., Ogawa, A., Morita, T., Kawaguchi,

Y., Kurihara, S. and Sakai, O. (1990) Am. J. Physiol. 258, F690–F696.
[20] Zhang, M.I.N. and O'Neil, R.G. (1996) Am. J. Physiol. 271, C1757–C1764.
[21] Vinay, P., Gougoux, A. and Lemieux, G. (1981) Am. J. Physiol. 241, F403–F411.
[22] Reid, J.M. and O'Neil, R.G. (2000) Biochem. Biophys. Res. Commun. 271, 429–434.
[23] Mochly-Rosen, D. (1995) Science 268, 247–251.
[24] Disatnik, M.-H., Boutet, S.C., Lee, C.H. and Mochly-Rosen, D. (2002) J. Cell Sci. 115, 2151–2163.
[25] Li, C., Fultz, M.E., Geng, W., Ohno, S., Norton, M. and Wright, G.L. (2001) Pflugers Arch. Eur. J. Physiol. 443, 38–47.
[26] Shirai, Y., Kashiwagi, K., Yagi, K., Sakai, N. and Saito, N. (1998) J. Cell Biol. 143, 511–521.