PKC-α-mediated remodeling of the actin cytoskeleton is involved in constitutive albumin uptake by proximal tubule cells

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Hryciw, Deanne H., Carol A. Pollock, and Philip Poronnik. PKC-α-mediated remodeling of the actin cytoskeleton is involved in constitutive albumin uptake by proximal tubule cells. Am J Physiol Renal Physiol 288: F1227–F1235, 2005.—One key role of the renal proximal tubule is the reabsorption of proteins from the glomerular filtrate by constitutive receptor-mediated endocytosis. In the opossum kidney (OK) renal proximal tubule cell line, inhibition of protein kinase C (PKC) reduces albumin uptake, although the isoforms involved and mechanisms by which this occurs have not been identified. We used pharmacological and molecular approaches to investigate the role of PKC-α in albumin endocytosis. We found that albumin uptake in OK cells was inhibited by the pan-PKC blocker bisindolylmaleimide-I and the isoform-specific PKC blockers Gö-6976 and 2',3',4',4''-hexahydroxy-1',1''-biphenyl-6,6''-dimethanol dimethyl ether, indicating a role for PKC-α. Overexpression of a kinase deficient PKC-α(K368R) but not wild-type PKC-α significantly reduced albumin endocytosis. Western blot analysis of fractionated cells showed an increased association of PKC-α-green fluorescent protein with the membrane fraction within 10–20 min of exposure to albumin. We used phalloidin to demonstrate that albumin induces the formation of clusters of actin at the apical surface of OK cells and that these clusters correspond to the location of albumin uptake. These clusters were not present in cells grown in the absence of albumin. In cells treated either with PKC inhibitors or overexpressing kinase-deficient PKC-α(K368R) this actin cluster formation was significantly reduced. This study identifies a role for PKC-α in constitutive albumin uptake in OK cells by mediating assembly of actin microfilaments at the apical membrane.

albuminuria; endocytosis; protein kinase C

THE KIDNEY PROXIMAL TUBULE plays a key role in absorbing albumin that is filtered across the glomerulus (8). Recent studies have estimated the concentration of albumin in the glomerular filtrate of humans to be as high as 3.5 mg/l (8, 29). The kidneys filter ~180 liters of blood/day, yet only 30 mg albumin are lost in the urine per day, indicating that the proximal tubule must reabsorb at least 600 mg albumin/day. This process occurs via a highly active and tightly regulated receptor-mediated pathway involving the albumin binding megalin/cubulin scavenger receptor (5, 8, 27). In addition to the receptor complex itself, the uptake of albumin appears to require the formation of a complex that includes the ion transporting proteins V-type H⁺-ATPase, Na⁺-H⁺ exchanger isoform 3 (NHE3), and the Cl⁻ channel CIC-5 (11, 20, 27). The V-type H⁺-ATPase plays a crucial role in the acidification of the endosomes/lysosomes required for the dissociation of albumin from the receptor (27), whereas NHE3 is thought to play a role in facilitating the initial acidification of the nascent endosome (11). CIC-5 is essential for albumin uptake. In the X-linked disorder Dent’s disease, mutations in CIC-5 lead to low-molecular-weight proteinuria/albuminuria (26). In addition to their roles as ion channels/transporters, however, both CIC-5 and NHE3 have large intracellular COOH-terminal domains that can interact with cytosolic proteins to link these proteins to the actin cytoskeleton and recruit regulate molecules (e.g., 15, 16, 21, 36). The best example of these interactions in epithelial cells involves the Na⁺-H⁺ exchange regulatory factor (NHERF) PDZ containing proteins that anchor NHE3 to the cytoskeleton via ezrin or α-actinin (21) and mediate the interaction with other regulatory proteins such as protein kinase C (PKC), protein kinase A, and phosphatidylinositol 3-kinase (24, 25, 34).

Formation of the nascent endosome after receptor-ligand binding involves complex interactions between the clathrin-coated pit, the endocytic complex, and the actin cytoskeleton (31). The actin microfilament network that makes up the cortical actin web is a dynamic structure. Assembly of the proteins comprising the endocytic complex requires actin as a support structure, while movement of the nascent endosome through the cortical actin web requires remodeling of the microfilament mesh to allow the endosome to pass this barrier (31). In cultured proximal tubule opossum kidney (OK) cells, disruption of the actin microfilament network with agents such as latrunculin A or cytochalasin D almost completely abolishes albumin uptake (9, 16), while the same agents appear not to affect basolateral endocytosis in Madin-Darby canine kidney cells (12). We have presented previously evidence for a crucial role of actin remodeling in albumin uptake. LIM kinase was used to inactivate the actin-depolymerizing activity of cofilin, and we found that this strongly inhibited albumin uptake in OK cells (16). Thus the intracellular COOH-terminus of CIC-5 binds cofilin to regulate the level of actin polymerization at the plasma membrane and allowing the nascent endosome to internalize when mature (16). Constitutive albumin uptake therefore requires tight regulation of actin polymerization to stabilize the endocytic complex and coordinate dissolution of the actin microfilaments in the vicinity of the nascent endosome (2, 31). We have identified one key protein involved in the disassembly process (i.e., cofilin; see Ref. 16); however, we

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have yet to identify the proteins that mediate actin stabilization during albumin uptake.

One group of proteins known to be involved in mediating changes in the actin cytoskeleton is the PKC family, and inhibition of pan-PKC results in a significant reduction in albumin uptake by OK cells (6). The PKC peptide is comprised of four conserved domains and five variable domains, with the NH2 terminus constituting the regulatory domain and the COOH terminus constituting the catalytic domain (18). Twelve isoforms of PKC have been identified to date, and these are divided into three groups (classical, novel, and atypical). The classical PKC isoforms are Ca2+ dependent and are activated by diacylglycerol (DAG) or phorbol esters (PKC-α, -βI, -βII, -γ); the novel PKC isoforms are Ca2+ insensitive and DAG sensitive (PKC-δ, -ε, -θ, and -η); and the atypical PKC isoforms are Ca2+ and DAG insensitive (PKC-ζ, -η, and -λ; see Ref. 35). PKC isoforms are implicated in numerous functions involving the regulation and remodeling of the actin cytoskeleton (19, 25, 31). Cells express multiple PKC isoforms, and, importantly, one isoform can have antagonistic effects on the function performed by another isoform (32). Indeed, it has recently been shown in T84 colonic carcinoma epithelial cells that basolateral membrane endocytosis involves both PKC-α and PKC-ε. In these cells, PKC-α acts to antagonize PKC-ε-mediated disassembly of F-actin, thereby stimulating basolateral fluid-phase endocytosis (32). Furthermore, PKC-α has recently been reported to bind to NHE3 via NH2RF-2 and thereby to contribute to agonist-mediated endocytosis of NHE3 (21). Based on the reduction of albumin uptake by nonspecific inhibitors of PKC and evidence for the involvement of PKC-α in endocytosis in epithelial cells, the current study was performed to determine whether PKC-α was involved in albumin uptake in OK cells.

MATERIALS AND METHODS

Cell culture. The OK cells (obtained from Dr. D. Markovich, University of Queensland) were maintained in DMEM/F-12 (GIBCO) medium supplemented with 10% FBS (GIBCO) and penicillin, streptomycin, and glucose (GIBCO). Cells were incubated at 37°C in 5% CO2 passed every 3–4 days at 80–90% confluence. For all experimental protocols, OK cells were seeded at confluence and maintained for 5 days to establish polarity. Before experimentation, cells were incubated for 48 h in serum-free 5 mM glucose DMEM/F-12 medium. This is a standard protocol for the culture of cells in preparation for measuring albumin endocytosis in OK cells (9, 15, 16).

Antibodies and plasmid transfection. A green fluorescent protein (GFP) antibody (Roche), FITC-phallolidin (Sigma-Aldrich), and TRITC-phallolidin (Calbiochem) were purchased. For Western blot detection of proteins, secondary antibodies conjugated to alkaline phosphatase were purchased from Bio-Rad. Latrunculin A was obtained from Biomol, whereas the other inhibitors were obtained from Calbiochem. Texas red conjugated to albumin (TR-albumin) was from Molecular Probes. Plasmids used were GFP (pEGFP-c1; Clontech), a wild-type (WT-PKC-α) or a dominant-negative kinase-deficient PKC-α (K368R) (provided by Dr. C. Schmitz-Peiffer, Garvan Institute of Medical Research, Sydney, Australia; originally from Prof G. Baier, Innsbruck, Austria), and GFP-tagged PKC-α (PKC-α-GFP; BD Biosciences). The transfection reagent Effectene (Qiagen) was used to transfect, following the manufacturer’s protocols.

Albumin uptake. Receptor-mediated albumin uptake was measured using previously published methods (9) with minor modifications (16). OK cells were preincubated in HEPES-buffered salt solution, pH 7.4 (HBSS), with the following: latrunculin A (1.5 μM for 60 min), cytochalasin D (10 μM for 40 min), bisindolylmaleimide-1 (BIM-1; 200 nM for 20 min), 2’,3’,3’,4’,4’,4’-hexahydroxy-1’,1’-biphenyl-6,6’-dimethanol dimethyl ether (HBDDE; 100 μM for 60 min), and Gö-6976 (1 μM for 60 min). Inhibitors were dissolved in DMSO and used at a final concentration of DMSO of 0.1%, a concentration that we have observed has no effect on albumin uptake under these experimental conditions, as has been previously reported (22). Cells were exposed to TR-albumin (50 μg/ml) in HBSS for 2 h at 37°C in the continued presence of the specific inhibitors. The cells were then washed in HEPES buffer, pH 6.0, and then lysed in MOPS buffer (20 mM MOPS, pH 7.4, with 0.1% Triton X-100) at 37°C for 45 min. The TR-albumin fluorescence was determined using a Fusion spectrophotometer (Hewlett Packard, Blackburn, Victoria, Australia) at 580 nm excitation and 630 nm emission wavelengths. TR-albumin uptake was adjusted for background and standardized to total cellular protein, and the amount of fluorescence per microgram cellular protein was calculated. For uptake studies in cells transfected with PKC-α, construct cells were transfected with 0.25 μg WT-PKC-α or PKC-α(K368R) plasmid per well. Transfected cells were incubated in DMEM/F-12 media for 5 days and then serum-free 5 mM glucose DMEM/F-12 medium for 2 days, following previously published protocols (9, 15, 16), and then albumin uptake was performed as described above.

Fractionation of OK cells. Cells transiently transfected with PKC-α-GFP were separated into membrane and cytosolic fractions following the previously described protocol (16). Aliquots (50 μg) of the membrane and cytosolic fractions were separated on 10% SDS-PAGE and Western blotted as described previously (16). PKC-α-GFP was then detected using an anti-GFP antibody and goat-anti-mouse conjugated to alkaline phosphatase secondary antibody. Densitometric analysis of the Western blot data was performed using Scion Image software.

Confocal analysis of OK cells. OK cells were seeded on glass coverslips and grown to confluence. OK cells were exposed to 1 mg/ml TR-albumin for 20 min, exposed to BIM-1 or Gö-6976 in the presence or absence of 1 mg/ml albumin, or transiently transfected with WT-PKC-α or PKC-α(K368R) and GFP. All cells were fixed in 4% paraformaldehyde, treated with 0.1% Triton X-100, and then stained with TRITC- or FITC-phallolidin for 2 h. Cells were analyzed by confocal microscopy. Z-series were taken by optically slicing the cells at 0.5-μm intervals. Cells were analyzed by confocal microscopy using a Bio-Rad Radiance 2000 confocal laser scanning microscope with 1.4 NA ×60 objective or Zeiss LSM 510 Meta confocal microscope with Plan-Apochromat ×63, 1.4 oil objective. GFP and FITC-phallolidin were excited at 488 nm, and emission was measured at 515 + 15 nm. TRITC-phallolidin and TR were excited at 543 nm, and emission was measured at 570 nm. The number of actin clusters per cell was calculated from the confocal images. Briefly, a series of images from confluent monolayers were selected from the different treatments. The number of actin clusters per cell was then calculated for all cells in the field of view. Where cells were transfected with the different plasmids, the number of actin clusters per cell was calculated only from GFP-expressing cells.

Statistical analysis. Statistical analyses of the data were performed using a two-tailed Student’s paired t-test with a P value <0.05 considered significant. All results are presented as means ± SE.

RESULTS

Pharmacological blockade of PKC. In the first instance, we confirmed the effect on TR-albumin uptake of inhibiting PKC with the nonspecific PKC blocker BIM-1, which inhibits all PKC isoforms. We found that in OK cells treated with BIM-1 (200 nM) there was a significant reduction in the uptake of TR-albumin to 64 ± 6% (n = 4; P < 0.01) of control values (Fig. 1). To identify the PKC isoform responsible for this effect, we repeated the experiments in the presence of HBDDE
A and 20 suggested a possible role for PKC-alpha (PKC-alpha). The fact that both HBDDE and Go-6976 blocked albumin uptake indicated that disruption of the cytoskeleton with these compounds resulted in TR-albumin uptake. Experiments were also performed with HBDDE reduced TR-albumin uptake to 62% (n = 4; P < 0.01) with cytochalasin D. The fact that both HBDE and Go-6976 blocked albumin uptake suggested a possible role for PKC-alpha.

Kinase-deficient PKC-alpha(K368R). To provide further evidence of a role for PKC-alpha in TR-albumin uptake in OK cells, a dominant-negative strategy was used. The PKC-alpha(K368R) mutant is kinase deficient; therefore, it is able to bind but not phosphorylate the substrate and hence acts as a dominant-negative PKC-alpha. Cells were transfected with either transfection reagent alone (control), WT-PKC-alpha, PKC-alpha(K368R), or GFP to indicate transfection efficiency. In cells expressing either WT-PKC-alpha or GFP, there was no significant difference in TR-albumin uptake compared with control mock-transfected cells (Fig. 2). Overexpression of PKC-alpha(K368R), however, significantly reduced the uptake of TR-albumin to 73 ± 4% of control levels (n = 4; P < 0.05; Fig. 2). This was also significantly different from the cells expressing either WT-PKC-alpha or GFP. It is important to note, as we have previously published (15, 16), that, under these conditions, the transfection efficiency of the OK cells is of the order of 50%; therefore, the ~27% reduction we observe in TR-albumin uptake in the total population of cells is a significant underestimate of the true reduction in TR-albumin uptake in cells overexpressing PKC-alpha(K368R).

Membrane association of PKC-alpha in response to albumin exposure. Activation of PKC is accompanied typically by an increased association of PKC with the cell membrane. To demonstrate a change in the levels of PKC-alpha associated with the membrane fraction, Western blots were performed on membrane and cytosol fractions from OK cells transfected with PKC-alpha-GFP. There was an increase in the amount of PKC-alpha-GFP associated with the membrane fraction after 10 and 20 min in OK cells in response to albumin (Fig. 3A). Densitometric analysis of the Western blots revealed a significant increase in the amount of PKC-alpha-GFP associated with the membrane fraction after 10 and 20 min (13 ± 3 and 25 ± 6% greater than control, respectively; n = 3; P < 0.05; Fig. 3B).

PKC, albumin, and the actin cytoskeleton. One of the known actions of PKC-alpha is to stabilize actin microfilaments (32). We therefore used confocal microscopy to determine the effects of albumin and PKC inhibition on the actin cytoskeleton. OK cells were labeled with TRITC-phalloidin after exposure to...
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Albinum with or without PKC inhibitors. In untreated cells, TRITC-phallolidin labeling showed that actin was located primarily in the basal and lateral membranes of the cells, giving the appearance of a “corona” at the apical surface in the x-y scan, with only a few clusters at the apical membrane (Fig. 4A). Treatment of cells with albumin (1 mg/ml) for 20 min led to an increase in the number of actin clusters at the apical surface of the cells, principally in clusters at the center of the apical membrane (Fig. 4B). This increase in TRITC-phallolidin staining was even more pronounced when the cells were observed in transverse section (z-scan: Fig. 4, C and D). Next, we performed colocalization experiments to determine if this clustering of actin was associated with the sites of albumin uptake. OK cells were exposed to TR-albumin for 20 min and then fixed and stained with TRITC-phallolidin. Confocal analysis revealed that the TR-albumin was distributed in puncta at the center of the apical membrane and that this overlapped with the actin clusters (Fig. 4E). These data indicate that, in the presence of albumin, there is a pronounced remodeling of the actin cytoskeleton at the apical surface, at the region where albumin uptake occurs. Analysis of the number of clusters per cell indicated that, in cells not exposed to albumin, there were few clusters (1.4 ± 1.1 clusters/cell; n = 30), whereas there was a significant increase after albumin exposure (21.1 ± 0.7; n = 30; P < 0.01; Fig. 4F).

We then investigated the effects of PKC inhibition on the increased actin polymerization observed in response to albumin. Treatment of the cells with BIM-1 had no effect on the distribution of actin in OK cells (Fig. 5C) compared with the control cells (Fig. 5A). In contrast, pretreatment with BIM-1 before exposure to albumin prevented the formation of actin clusters (Fig. 5D) that was observed in cells exposed to albumin alone (Fig. 5B). Similar effects were observed in cells exposed to Gö6976 and albumin (Fig. 5, E and F). Analysis of the number of actin clusters showed a low level in control (3.3 ± 0.4 clusters/cell; n = 30) that was significantly increased when cells were exposed to albumin (18.9 ± 1.3 clusters/cell; n = 30; P < 0.01). In cells exposed to BIM-1 or Gö6976, there was a similar number of actin clusters (3.2 ± 1.5 and 5 ± 0.2/cell, respectively; n = 30) compared with control cells (Fig. 4G). However, in cells treated with BIM-1 or Gö6976 and exposed to albumin, there was no increase in the number of clusters in response to albumin (3.97 ± 0.6 and 4.2 ± 1.2/cell, respectively; n = 30; Fig. 4G). These data suggest that the actin cluster formation in response to albumin is dependent on PKC and, in particular, PKC-α.

Kinase-deficient PKC-α and the actin cytoskeleton. To demonstrate a specific role for PKC-α in the albumin-mediated changes in the actin cytoskeleton, we used a dominant-negative transfection strategy. OK cells were transfected with WT-PKC-α or PKC-α(K368R) together with GFP to identify the transfected cells, and the effects of albumin on actin distribution were investigated. Cells overexpressing the PKC-α constructs were exposed to albumin for 20 min and then fixed and labeled with TRITC-phallolidin. In cells exposed to albumin, there was the characteristic increase in apical clusters of actin in cells overexpressing GFP and WT-PKC-α, similar to that observed in nontransfected cells (Fig. 6, A-D). In contrast, in cells overexpressing PKC-α(K368R), the clustering of actin was effectively abolished (Fig. 6, E and F). Analysis of the number of clusters indicated that cells transfected with GFP or WT-PKC-α indicated that the levels of clusters were similar between the two (16.86 ± 1.2 and 17.23 ± 0.2, respectively; Fig 6G). However, there was a significant reduction in the number of clusters in cells transfected with GFP and PKC-α(K368R) (3.03 ± 0.5, P < 0.01; Fig. 6G). Taken together, these data support the hypothesis that suppression of normal PKC-α activity in OK cells by PKC-α(K368R) leads to a reduction in albumin uptake by preventing normal actin microfilament assembly at the apical membrane.

DISCUSSION

Albumin uptake by OK cells is known to be inhibited by blockers of PKC (9); however, the molecular basis of this inhibition remains unclear. In the current study, we present evidence of a role for the PKC-α isoform in constitutive albumin uptake by a mechanism involving the clustering of actin microfilaments in the regions of the apical membrane where albumin uptake occurs. It is clear that an intact and dynamic actin cytoskeleton is essential for receptor-mediated albumin uptake by the proximal tubule (9, 16). The formation of an actin microfilament network at the endocytic site provides a structural foundation for the recruitment scaffold proteins (such as ezrin-radixin-moesin-binding proteins) that in turn link the proteins involved in endocytosis and its regulation into a macromolecular complex (33). We postulate that, in the proximal tubule, complexes formed between NHERFs, NHE3, and the cytoskeleton (36) may facilitate albumin uptake by forming part of the scaffold/signaling complex required for albumin uptake. In the current study, we highlight the importance of such actin-based scaffolds for albumin uptake in OK cells by showing that albumin induces the formation of clusters of apical actin microfilaments at the sites of albumin uptake. These structures are absent in cells grown in serum (albumin)-free medium and also appear when cells are exposed to lower concentrations of albumin (i.e., 10 and 100 µg/ml; data not shown). We postulate that these actin clusters act as the foundation to which scaffold proteins bind and assemble the macromolecular complex required for albumin uptake. It is significant that the location of the clusters of actin at the center of the apical membrane closely resembles the distribution of VSV-tagged NHE3 in OK cells stably expressing this epitope-tagged NHE3 (1).

The inhibition of albumin uptake by nonspecific PKC blockers such as BIM-1 probably involves effects on multiple PKC isoforms (6). In the current study, the involvement of PKC-α in the assembly of the actin clusters in response to albumin uptake has been defined by using both pharmacological blockade and overexpression of WT and kinase-deficient PKC-α. The roles of PKC-α in the remodeling of the actin cytoskeleton during cell motility, phagocytosis, or neurite outgrowth are well known (4, 14), as well as the ability of PKC-α to regulate actin-associated proteins (13). The fact that overexpression of WT PKC-α does not significantly enhance albumin uptake or initiate the formation of apical actin clusters in the absence of albumin indicates that other factors/proteins are required and that PKC-α is one key element of the complex.

The current study does not identify the upstream or downstream effectors of PKC-α. For example, it is well known that PKC-α binds to phospholipase D, which is functionally associated with actin-based microfilament cytoskeleton (7). PKC-α
Fig. 4. Albumin causes formation of apical actin clusters. OK cells were incubated with 1 mg/ml albumin for 20 min and actin stained with TRITC-phalloidin for confocal microscopy. A: control cells showing very few actin clusters at the apical membrane. B: albumin induced the formation of clusters of actin at the center of the apical membrane in each cell. C: Z-axis scan of control cells showing low level of actin staining at the apical membrane. D: z-axis scan of cells exposed to albumin showing a pronounced increase in actin clusters at the apical membrane. E: TR-albumin, OK cells labeled with TR-albumin for 20 min showing uptake; FITC-actin, FITC-phalloidin labeling showing clusters of actin at the apical membrane in response to TR-albumin; Merge, merged images showing colocalization of actin clusters and TR-albumin (yellow); zoom, enlarged cell demonstrating colocalization of actin clusters and TR-albumin (yellow) indicated with arrows. All images are representative of randomly selected fields of view of confluent monolayers from 3 separate experiments, with the total number of cells equal to 30 (scale bar = 10 μm). F: analysis of the number of actin clusters per cell showed that there was a significant increase in the number of actin clusters per cell in response to albumin (*P < 0.01; n = 30).
Fig. 5. PKC inhibition prevents albumin-induced actin clusters. OK cells were preincubated with PKC inhibitors and treated with albumin (1 mg/ml) for 20 min, and the actin cytoskeleton was stained with TRITC-phalloidin for confocal microscopy. Cells treated with PKC inhibitors did not show an increase apical actin cluster in response to albumin. A: control cells. B: cells exposed to albumin. C: control cells treated with BIM-1. D: BIM-1-treated cells exposed to albumin. E: control cells treated with Go-6976. F: Go-6976-treated cells exposed to albumin. All images are representative of randomly selected fields of view from 3 separate experiments (scale bar = 10 μm). G: analysis of the number of actin clusters per cell. Albumin caused a significant increase in clusters per cell (*P < 0.01; n = 30). Inhibition of PKC with BIM-1 or Go-6976 prevented any increase in the number of clusters per cell in response to albumin.
Fig. 6. Kinase dead PKC-α(K368R) prevents albumin-induced increase in actin clusters. OK cells were transiently cotransfected with WT-PKC-α or PKC-α(K368R) together with GFP to identify the transfected cells. The cells were exposed to albumin (1 mg/ml) for 20 min and then stained with TRITC-phalloidin for confocal analysis. The TRITC-phalloidin images are shown in A, C, and E, and the corresponding GFP images are in B, D, and F. In A, C, and E, the asterisks (*) indicate the cells expressing GFP (transgene-expressing cells) in B, D, and F. A: cells exposed to albumin showing the presence of actin clusters in untransfected and GFP expressing cells. C: cells expressing WT-PKC-α (*) also showing the formation of actin clusters in response to albumin. E: cells expressing PKC-α(K368R) (*) fail to show the formation of actin clusters after treatment with albumin. All images are representative of at least 10 different fields of view from 3 separate experiments (scale bar = 10 μm). G: analysis of the number of actin clusters per cell showed that cells exposed to albumin expressing PKC-α(K368R) and GFP had a significantly reduced number of clusters per cell (*P < 0.01; n = 30) compared with cells expressing GFP or WT-PKC-α and GFP.
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can also act on small GTPases that in turn alter actin dynamics (e.g., see Ref. 12). It has recently been reported in colonic carcinoma T84 epithelial cells that basolateral membrane endocytosis involves both PKC-α and PKC-ε. In these cells, PKC-α acts to antagonize PKC-ε-mediated disassembly of F-actin, thereby stimulating basolateral fluid-phase endocytosis (32). It is interesting to note that PKC activity has been shown to dephosphorylate cofilin, an effect that would be expected to increase actin disassembly (37). This effect, however, is independent of PKC-α since Gö-6976 has no effect on the levels of cofilin phosphorylation (37). Our data are thus consistent with a similar counterregulatory role for PKC-α in constitutive albumin uptake by OK cells by stabilizing actin microfilaments and thereby antagonizing the activity of another PKC isozyme or protein involved in cofilin-mediated actin disassembly.

The kinetics of albumin uptake have been well characterized in OK cells, with the linear phase of albumin uptake lasting ~30 min (10). Our data show an increased association of PKC-α with the membrane fraction in proximal tubule cells with a similar time course. Based on these data that show dramatic changes in actin at the apical membrane within 20 min, we postulate that the time taken to reach maximum albumin uptake is required for the de novo assembly of the actin substructure and the recruitment of the proteins required to form the endocytic complex. A similar rapid time frame has been shown for the translocation of PKC-α in LLC-PK1 cells treated with dopamine (30). Treatment of OK cells with dopamine, which increases cAMP levels, leads to a rapid downregulation of NHE3 activity resulting from increased clathrin-mediated endocytosis of NHE3 protein (3, 17). In a more recent study, a role for PKC-α in mediating the endocytosis of NHE3 has also been implicated in PS120 fibroblasts (25). This study concluded that the effect of PKC-α may be have been the result of the increased endocytosis of NHE3 and reduced numbers at the cell surface in response to calcium. This raises the important question as to whether NHE3, or proteins associated with it, undergo PKC-α-mediated endocytosis independent of other endocytosed proteins (25).

This current study further highlights the role that albumin plays in assembling of the macromolecular complex required for albumin endocytosis. Previously, we have demonstrated that albumin increases cell surface expression of NHE3 (23) and CIC-5 (15), proteins essential for albumin uptake, and upregulates levels of proteins that are involved in the endocytic complex, such as the ubiquitin ligase Nedd4–2 (15). We now demonstrate a role for PKC-α in mediating the changes in the cytoskeleton required for the optimal constitutive uptake of albumin. It must be stressed that albumin uptake by the proximal tubule is a high-capacity and constitutive process that involves a transmembrane receptor complex (megalin/cubulin) with a large COOH-terminal domain that contains numerous as yet uncharacterized regulatory motifs (28) and that albumin acts as an agonist at this receptor. Our data provide further evidence to show that, during the uptake of albumin, the cytoskeleton and membrane undergo significant remodeling that depends on agonist-mediated changes in the distributions of key regulatory proteins. The recruitment of PKC-α to the plasma membrane may involve interactions with the epithelial scaffolds, such as NHERF-2 (25). These data underscore the complexity of the molecular events that underlie constitutive albumin uptake by the proximal tubule and the need to elucidate these mechanisms to understand the molecular basis of pathophysiological states.

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REFERENCES


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