Dephosphorylation of the Cadherin-associated p100/p120 Proteins in Response to Activation of Protein Kinase C in Epithelial Cells*

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Protein kinase C signaling pathways have been implicated in the disruption of intercellular junctions, but mechanisms are not clear. p100 and p120 are members of the Armadillo family of proteins and are localized to cellular adherens junctions. In strain I Madin-Darby canine kidney cells, protein kinase C activation leads to disruption of tight junctions and an increase in permeability of cell monolayers. We show that this permeability increase is accompanied by dephosphorylation of p100/p120 on serine and threonine residues. The dephosphorylation of these proteins can also be induced by the kinase inhibitors staurosporine, KT5926, and Gö 6976. Treatment of cells with phosphatase inhibitors induced hyperphosphorylation of p100 and p120. Thus, p100 and p120 participate in a regulatable cycle of serine/threonine phosphorylation and dephosphorylation. Protein kinase C must act, directly or indirectly, by perturbing this phosphorylation cycle, by inhibition of a p100/p120 kinase and/or activation of a phosphatase. These data clearly show that p100 and p120 are targets of a novel protein kinase C signaling pathway. Dephosphorylation of these proteins precedes the permeability increase across epithelial cell monolayers seen in response to phorbol esters, raising the possibility that this pathway may play a role in the modulation of intercellular junctions.

Intercellular junctions are important sites of regulation. Cells recognize and adhere only to their correct neighbors. Once contact is made, they signal each other to stop growing and interact to form a cooperating unit. In certain physiological situations, such as wound healing or tissue morphogenesis, cell junctions can be disrupted in a controlled manner to allow cell migration (for a review, see Ref. 1). Conversely, inappropriate disruption of junctions can have unfortunate consequences. For example, loss or mutation of certain components of junctions in transformed cells results in increased invasiveness and metastatic potential (2–5).

Cell-cell junctions are particularly well developed in epithelial cells. These have discrete, specialized regions of cell-cell adhesion comprising the tight junction, which forms the main barrier to paracellular traffic, the adherens junction, and the desmosome. Evidence suggests that the adherens junction is an important site of regulation and may be an important target for signaling pathways. Furthermore, other junctions, such as the tight junction, are dependent on prior formation of the adherens junction (6, 7) and therefore indirectly subject to modulation of adherens junction function.

The basic architecture of adherens junctions is well established. They are formed by Ca2+-dependent homotypic interactions between cadherin molecules on adjacent cells (8, 9). The cadherins are linked to the actin-based cortical cytoskeleton via α-, β-, and γ-catenins, which bind to the cytoplasmic domain of cadherins and are essential for adhesion (10–12). α-Catenin is homologous to vinculin and so may provide the link to the actin cytoskeleton (13, 14). β-Catenin and γ-catenin (plakoglobin) are members of the Armadillo family of proteins (15–18). Proteins comprising this family contain copies of a 42-amino acid repeat motif that was first identified in the Drosophila segment polarity gene product Armadillo (19–21).

A number of different signaling pathways can regulate intercellular junctions. Several studies have suggested that tyrosine phosphorylation plays a role in controlling junctional integrity, and evidence has pointed to components of the adherens junction as possible molecular targets. The tyrosine kinases c-Yes, c-Src, and c-Lyn have been found at adherens junctions (22), where levels of phosphotyrosine can, under certain conditions, become elevated (22–24). Transfection of epithelial cells with oncogenic Src or Ras leads to tyrosine phosphorylation of components of the adherens junction, and this correlates with loss of cell-cell adhesion (4, 25–27). In cells with well developed junctions, tyrosine phosphatase inhibitors also lead to increased tight junction permeability in association with tyrosine phosphorylation of junctional proteins (28).

p120, like β-catenin, is a member of the Armadillo family of proteins, having 11 copies of the 42-amino acid Armadillo repeat (29). It was first discovered as a protein whose phosphorylation on tyrosine residues correlated with transformation in cells transfected with pp60-src (30, 31). p120 is also tyrosine-phosphorylated following stimulation of cells by the growth factors epidermal growth factor, colony-stimulating factor, and platelet-derived growth factor (32, 33). Recently, p120 has been shown to be localized to adherens junctions via interaction with cadherins, and a number of different, closely related, isoforms have been identified (34–37). The association of p120 with cadherins is further intriguing evidence that cell-cell adhesion may be regulated by signaling pathways.

Protein kinase C (PKC),1 a serine/threonine kinase, has for some time been implicated in the regulation of cell-cell adhesion, but although much is known about the kinase itself (38), in molecular terms its effects on adhesion are very poorly understood. PKC plays a role in assembly of tight junctions (39–41), but its activation can also lead to disruption of those of established cell monolayers, causing an increase in paracellular permeability (42–44). PKC activation has also been strongly implicated in cell growth control, which also involves alterations in cell-cell adhesion. The targets of the PKC signal-
ing pathway and how changes in cell adhesion are achieved are not known. In this study, we report that components of the adherens junction, p120 and the related protein p100, are major targets of a PKC-mediated signaling pathway in epithelial cells and thus may play a role in modulation of cell-cell adhesion.

**EXPERIMENTAL PROCEDURES**

**Cells**—Strain I MDCK cells (epithelial cells derived from canine kidney) were provided by Barry Gumbiner (Memorial Sloan-Kettering Cancer Center, New York). Strain II MDCK cells were from the American Type Culture Collection, LCC-PK, epithelial cells derived from porcine kidney and Caco-2 cells (epithelial cells derived from a human colonic tumor) were obtained from the European Collection of Animal Cell Cultures (Salisbury, UK). All cells were maintained at 37 °C under 5% CO2 in humidified air. MDCK cells were maintained in minimal essential medium containing 10% fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. Caco-2 cells were maintained in minimal essential medium containing 15% fetal calf serum, 1% nonessential amino acids, and 1 µg/ml bovine insulin. LCC-PK, cells were grown in M199, 10% fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. The supernatant was precleared with 10% (w/v) protein A-Sepharose. The beads were washed five times with lysis buffer, and the bound protein was solubilized in SDS-sample buffer.

**Phosphatase Assays**—Phosphatases were assayed as described previously (45). The [32P]phosphate incorporated into the proteins was measured by using von Kostyuk and Twarog’s (50) and 100 µCi/ml [32P]orthophosphate (Amersham). Cells were treated with or without 200 nM PDB for 30 min and then lysed into TDS buffer and immunoprecipitated with p120 antibodies as described above.

**PKC Activation Alters the Electrophoretic Mobility of p100**

Whole cell lysates or immunoprecipitates were electrophoresed in 10% SDS-PAGE gels, then transferred to nitrocellulose filters (Hybond ECL; Amersham), which were S-stained and then blocked for 2 h at 37 °C with 1% bovine serum albumin in PBS containing 0.05% Tween 20. Filters were then probed with p120 antibody at a dilution of 1:2500. After washing, filters were incubated with horseradish peroxidase-conjugated anti-mouse antibody (Amersham), at a dilution of 1:5000. The filters were then extensively washed in PBS, 0.5% Tween 20, and immunoreactive bands were detected by enhanced chemiluminescence (Amersham) following the manufacturer's instructions.

**Immunofluorescence**—Cells were fixed with 3% paraformaldehyde made up in PBS containing 0.5 mM CaCl2 and 0.5 mM MgCl2. Fixed cells were washed and then permeabilized by ice-cold PBS (containing 0.9 mM CaCl2 and 0.5 mM MgCl2) before the addition of 0.5 µl of appropriate fluorescent antibody. The filters were then gently scraped, and the lysate was removed and centrifuged at 110 °C for 1 h in 5.7 µl HCl to release PAs. After lysis preparation, two-dimensional PAA separation and detection was carried out as described previously (46).

**Resistance and Paracellular Flux Measurements**—Resistance measurements of cells grown on 6.5-mm transwells were taken using a Millicell-ERS resistance system (Millipore Corp.). For paracellular flux measurements, 1 µCi of [3H]sucrose in 25 µl of PBS was added to the apical side of cells grown on 6.5-mm transwells. Cells were incubated at 37 °C, shaken at 10-min intervals, and all the medium from the basolateral chamber was removed for scintillation counting at the times indicated.

**RESULTS**

**PKC Activation Alters the Electrophoretic Mobility of p100 and p120**—MDCK cells are a kidney-derived epithelial cell line. Two strains exist: strain I, which develop high electrical resistance across monolayers in culture due to extremely well developed tight junctions; and strain II, which have lower electrical resistance. (47). Most of the following experiments were carried out using strain I cells. These cells were grown on polycarbonate filters, allowing measurement of resistance across cell monolayers. Cells with a resistance of 2000–4000 ohm-cm2 were used. Consistent with previous findings (42), the addition of 200 nM PDB caused a rapid decrease in resistance (in our hands, resistance dropped to 58.3 ± 7.7% of control after 30 min of incubation with PDB). As part of a series of experiments analyzing the effects of PKC activation on cell junction proteins, whole cell lysates from MDCK I cells treated with or without 200 nM PDB for 30 min were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with antibodies to the adherens junction proteins p100/p120 (Fig. 1). Whereas the p100 and p120 from untreated cells were visualized as rather diffuse bands, these proteins from PDB-treated cells appeared as tighter, faster migrating bands. In contrast, the mobility and the amount of E-cadherin were unaffected.
PKC and p100/p120

The migration of p100 and p120 during SDS-PAGE is increased following PDB treatment of MDCK I cells. Cells treated for 30 min with either 200 nM PDB or vehicle (Me2SO at a 1:1000 dilution) were lysed into Laemmli sample buffer and separated by SDS-PAGE using 6% polyacrylamide gels. Proteins were transferred to nitrocellulose and probed with antibodies to p100/p120 (A) and then reprobed with antibodies recognizing either E-cadherin (B) or paxillin (C). PDB treatment caused p100 and p120 to migrate as faster, tighter bands and had no effect on E-cadherin but retarded the mobility of paxillin. Other experiments indicated that β-catenin mobility apparently was not affected by PDB treatment.

(Fig. 1B), indicating equal protein loading, and, as previously reported for fibroblasts (48), the mobility of paxillin was reduced (Fig. 1C).

PKC activation leads to increased paracellular permeability in other epithelial cell lines (43, 44). We investigated whether p100 and p120 isolated from these cells showed altered mobility during electrophoresis. Resistance of Caco-2 and LLC-PK1 monolayers decreased following PDB stimulation (as previously reported). Paracellular flux of [14C]sucrose is also increased (Fig. 2, A and C). MDCK II cells did not show an appreciable drop in resistance in response to PDB, but did show an increase in paracellular flux across cell monolayers (Fig. 2E), indicating some disruption of cell-cell junctions. In all of these cell lines, stimulation of PKC led to increased mobility of p100/p120 (Fig. 2, B, D, and F).

PKC Dependence and Reversibility—Pretreatment of MDCK I cells with the specific PKC inhibitor bisindolylmaleimide I (49) or Ro 31–8425 (50) for 5 min prior to PDB addition completely abolished the p100/p120 band shift (Fig. 3A). Bisindolylmaleimide V, a member of the bisindolylmaleimide class that does not inhibit PKC, was unable to block the PDB-induced band shift. Analysis of dose dependence showed that PDB was effective at concentrations between 30 and 100 nM (Fig. 3B). PKC activation leads to increased paracellular permeability in other epithelial cell lines (43, 44). We investigated whether PKC and p100/p120...

PDB Induces Dephosphorylation of p100 and p120—We investigated the possibility that the altered pattern of migration during electrophoresis of p100 and p120 following treatment with PDB was due to a change in the phosphorylation state of these proteins. MDCK I cells were metabolically labeled with [32P]orthophosphate and treated with or without PDB, and the p100 and p120 were isolated by immunoprecipitation. This immunoprecipitation was carried out in TDS lysis buffer to remove any associated proteins from p100 and p120. Protein was separated by SDS-PAGE, transferred to nitrocellulose, and autoradiographed (Fig. 5A). The level of phosphate present in p100 and p120 from untreated cells was compared with that from PDB-treated cells by densitometric scanning of the resulting autoradiograph (Fig. 5B). Immunoblotting of these filters,...
followed by scanning, allowed the phosphate signal to be normalized in relation to the amount of protein analyzed. (Fig. 5, A and B). This corrected for small errors due to slight differences in protein recovery. It was calculated that the [32P]phosphate content of p100 and p120 was reduced by approximately 40% following PDB treatment. The dephosphorylation of p100 and p120 is specific, since there was no change either in the phosphorylation of E-cadherin immunoprecipitated from the same cell lysates or in the phosphorylation state of proteins in whole cell extracts (data not shown). This rules out the extremely unlikely possibility that the effect on p100/p120 was an artifact due to nonspecific effects of PDB on labeling of the γ-phosphate of the cellular ATP pool.

PAA analysis of p100/p120 from MDCK I cells showed that in resting cells these proteins contained mainly phosphoserine, with a small amount of phosphothreonine and no detectable phosphotyrosine (Fig. 5C). Following the addition of PDB, there was a reduction in the level of phosphorylation of both serine and threonine residues. Densitometric analysis of the autoradiographs of the PAA analysis revealed a reduction in phosphate signal of approximately 40% (data not shown), agreeing with the values obtained from the whole protein phosphate labeling experiments.

Effect of Kinase Inhibitors and Phosphatase Inhibitors on p100 and p120—To bring about dephosphorylation of p100/p120, PKC activation must lead to net kinase inhibition and/or phosphatase activation. Since the effect on p100/p120 is rapid and can easily be reversed, it is clear that p100/p120 is capable of cycling between phosphorylated and lesser phosphorylated forms, subject to the action of kinase(s) and phosphatase(s). To test this further, MDCK I cells were treated with kinase or phosphatase inhibitors in an attempt to perturb this cycle. Thus, 100 nM staurosporine, a kinase inhibitor (53), induced a p100/p120 band shift similar to that seen in response to PDB (Fig. 6A). This would be consistent with direct inhibition of a p100/p120 kinase by staurosporine. However, staurosporine inhibits many kinases, and it could be argued that its effects on p100 and p120 were indirect. Screening a variety of kinase inhibitors showed that the addition of KT5926, a staurosporine derivative that has a more restricted number of targets (54,
The results of the scanning of the autoradiographs shown in panel A of phosphate per unit of protein could be calculated. p100/p120 protein was determined by densitometry. Thus, the amount of phosphate in p100/p120 from control cells, relative to that from PDB-treated cells, was determined by densitometric scanning. To normalize for slight differences in protein loading, the same loading was used for all samples. After excision, the PAAs were separated by two-dimensional electrophoresis. p100/p120 from control cells contained mainly phosphoserine (S) and some phosphothreonine (T) but no detectable phosphotyrosine (Y). Following PDB treatment, both phosphoserine and phosphothreonine levels were reduced. However, some phosphothreonine and no detectable phosphotyrosine remained in the treated samples.

Activation of PKC has been shown to increase the permeability of tight junctions in established epithelial cell monolayers. Since adherens junction are necessary for the maintenance of tight junction integrity, we investigated whether components of the adherens junction are targets of PKC signaling pathways. This study shows that the adherens junction proteins p100 and p120 are targets for a PKC-mediated signal transduction pathway in epithelial cells. In unstimulated MDCK I cells, p100 and p120 are phosphorylated primarily on serine residues, with some phosphothreonine and no detectable phosphotyrosine. Approximately 40% of these residues are dephosphorylated within 5 min of the addition of PDB to cells. This loss of phosphate is presumably sufficient to dramatically alter

**Fig. 5.** The change in phosphorylation of p100/p120 following PDB treatment. A, following overnight labeling with 32P orthophosphate. MDCK I cells were exposed for 30 min to 200 nM PDB or vehicle only and lysed in TDS buffer. p100 and p120 were immunoprecipitated and blotted, and labeled protein was visualized by autoradiography. The level of phosphate in p100 and p120 from control cells, relative to that from PDB-treated cells, was determined by densitometric scanning. To normalize for slight differences in protein loading, the same filters were then probed with p100/p120 antibody and the level of p100/p120 protein was determined by densitometry. Thus, the amount of phosphate per unit of protein could be calculated. Panel B shows the results of the scanning of the autoradiographs shown in panel A, with 32P and protein signal measured in OD/mm/mm. Pn indicates phosphate level normalized for protein. In this experiment, approximately 40% of the phosphate in p100/p120 was lost following PDB treatment. The results from seven independent sets of such experiments showed that following PDB treatment, the phosphate content of p100/p120 was reduced to 58 ± 11% (mean ± S.D.) of that of untreated cells. Panel C, PAA analysis. Following phosphate labeling, immunoprecipitation, and visualization of labeled p100/p120 as above, the levels of p100/p120 protein were determined by immunoblotting, and in this case it was ascertained that equal amounts of protein from PDB-treated and untreated cells were used for the PAA analysis. The p100/p120 bands were excised, and the PAAs were separated by two-dimensional electrophoresis. p100/p120 from control cells contained mainly phosphoserine (S) and some phosphothreonine (T) but no detectable phosphotyrosine (Y). Following PDB treatment, both phosphoserine and phosphothreonine levels were reduced.

**Fig. 6.** Effect of kinase inhibitors and phosphatase inhibitors on p100/p120 electrophoretic mobility. MDCK I cells were treated with staurosporine (100 nM), KT5926 (1 µM), Go 6976 (1 µM), or Go 7874 (1 µM) for 60 min, and p100 and p120 were analyzed by immunoblotting (A). The addition of the phosphatase inhibitor okadaic acid (1 µM) or calyculin A (100 nM) for 60 min caused an upward band shift of p100/p120 (B), consistent with increased phosphate content.

**Discussion**

The E-cadherin-associated Pool of p100/p120 Is Dephosphorylated—It has been shown that although some of the p100/p120 in cells is associated with adherens junctions, there is also a large pool that is not complexed with cadherins, at least in immunoprecipitation analysis of Triton X-100-solubilized proteins (see, for example, Refs. 34 and 35). To determine which of these p100/p120 pools are subject to dephosphorylation, MDCK I cells were treated with PDB, extracted using Triton lysis buffer (which preserves protein–protein interactions), and immunoprecipitated with antibodies recognizing E-cadherin. This procedure serves to isolate only that portion of the cellular p100/p120 that associates with cadherin. Following SDS-PAGE and transfer to nitrocellulose, the p100 and p120 present in E-cadherin immunoprecipitates were detected by immunoblotting (Fig. 7A). It can be seen that the E-cadherin-associated p100/p120 is dephosphorylated in response to PDB. Immunoblot analysis of the non-E-cadherin-associated p100/p120 pool revealed that this too was subject to dephosphorylation (data not shown). There was no change in the amount of p100 and p120 present in E-cadherin immunoprecipitates after the addition of PDB. [35S]Methionine labeling followed by immunoprecipitation of p100/p120 under conditions that preserved protein–protein interactions confirmed that there was no change in the association of p100 and p120 with E-cadherin, α-catenin, or β-catenin (Fig. 7B). In addition, immunofluorescence studies in MDCK I cells revealed no apparent change in the localization of p100 and p120 after PDB treatment (Fig. 7, C and D).
PKC and p100/p120

The mobility of these proteins during SDS-PAGE. The dephosphorylation can be reversed rapidly by subsequent inhibition of PKC by bisindoylmaleimide I. These data suggest that in resting epithelial cells, the level of p100/p120 phosphorylation is kept constant by the opposing actions of a serine/threonine phosphatase and a corresponding kinase (see Fig. 8). Upon the addition of PDB, p100 co-migrates with β-catenin, so this diffuse band seems to disappear. Immunofluorescence studies in MDCK I cells revealed no obvious difference in cellular localization of p100/p120 following PDB-treatment. Panel C shows p100/p120 in control cells; panel D shows p100/p120 in PDB-treated cells. Bar, 40 μm.

Fig. 7. Cadherin-associated p100/p120 is dephosphorylated in response to PDB. MDCK I cells treated for 30 min with either 200 nM PDB or vehicle were extracted into Triton lysis buffer, and the adherens junction complex was immunoprecipitated with antibodies recognizing E-cadherin. Following SDS-PAGE and transfer to nitrocellulose, the immunoprecipitates were probed with antibodies to p100/p120 (A). E-cadherin-associated p100 and p120 were dephosphorylated in response to PDB. Immunoprecipitation (IP) of p100 and p120 from [35S]methionine-labeled cells, under conditions where protein-protein interactions were maintained, showed that the association of p100 and p120 with other components of the adherens junction was unchanged following PDB treatment (B). The locations of E-cadherin (E), α-catenin (α), and β-catenin (β) are indicated. The presence of p100, seen as a diffuse band in untreated cells, is indicated by the open arrowhead. Upon the addition of PDB, p100 co-migrates with β-catenin, so this diffuse band seems to disappear. Immunofluorescence studies in MDCK I cells revealed no obvious difference in cellular localization of p100/p120 following PDB-treatment. Panel C shows p100/p120 in control cells; panel D shows p100/p120 in PDB-treated cells. Bar, 40 μm.

It is evident that PKC must be exerting its effects on p100/p120 via a signaling pathway that comprises at least two steps. PKC may act to directly inhibit the p100/p120 kinase or trigger a multisite signaling pathway leading to its inactivation. Alternatively, or additionally, PKC may cause p100/p120 dephosphorylation by stimulating phosphatase activity. At present, it is impossible to deduce whether the phosphatases inhibited by okadaic acid and calyculin A (protein phosphatase 1 and protein phosphatase 2A (for a review, see Ref. 59)) are those responsible for the dephosphorylation in response to PDB and staurosporine. The only definite conclusion that can be drawn is that both p100 and p120 are substrates for these enzymes. The identity of the p100/p120 kinase also remains to be determined. One potential candidate is glycogen synthase kinase-3β. This kinase has been shown to be involved in signaling pathways that affect proteins of the Armadillo family (see, for example, Refs. 60–62). Glycogen synthase kinase-3β is known to be phosphorylated and inhibited by PKC (63), and PKC has recently been implicated in wingless signaling (64).

An important feature of our results is that a mobility shift of p100 and p120, consistent with dephosphorylation, occurred in response to PDB treatment in a variety of epithelial cell lines, which suggests that this PKC-triggered dephosphorylation pathway could be a general feature of epithelial cells. The magnitude of the effect varied between different cell types; a clear mobility shift was seen in MDCK I, MDCK II, and LLC-PK1 cells, but a relatively small change in p100/p120 mobility occurred in response to PDB in Caco-2 cells. However, the p120 isoforms from untreated Caco-2 cells already migrate as rather tight bands during electrophoresis. Perhaps these proteins already exist in a relatively dephosphorylated form, possibly explaining the less striking effect of PDB treatment.

The identification of a p100/p120 serine/threonine phospho-

Fig. 8. Schematic representation illustrating how PKC activation may lead to dephosphorylation of p120. Note that the closely related p120 isoform, p100, would be subject to the same cycle. In resting cells, p120 is phosphorylated on serine and threonine, the level of phosphate being maintained by the opposing actions of a serine/threonine kinase and a phosphatase. PDB activation of PKC may cause it to directly phosphorylate the p120 kinase, switching it from an active (kinase*) form to an inactive (kinase) form as represented here. This would lead to p120 dephosphorylation. Equally, PKC could act to inhibit the p120 kinase via a multistep signaling pathway. Alternatively, PKC could somehow increase the activity of the serine/threonine phosphatase, which would also cause dephosphorylation of p120. This contrasts with the previously known tyrosine phosphorylation cycle of p120; in resting cells, there is little or no tyrosine phosphorylation of p100/p120, but following stimulation of Src, or perhaps other tyrosine kinases, p120 is phosphorylated on tyrosine.
in the subcellular localization of p100/p120 following PDB stimulation, and immunoprecipitation studies showed that dephosphorylated p100/p120 remained associated with the cadherin-catenin complex. Thus, there would appear to be no gross disruption of the architecture of adherens junctions following PKC activation.

Adherens junctions have been shown to regulate the function of tight junctions (6, 65), raising the possibility that p100/p120 dephosphorylation could be important in the disruption of tight junctions seen in response to PKC activation. Indeed, MDCK I, LLC-PK1, and Caco-2 cell monolayers all showed increased permeability following PKC activation due to disruption of tight junctions (42–44). It is possible that PKC signals to the tight junction via effects on the adherens junction. p100/p120 dephosphorylation may be a necessary intermediate step in the signaling pathway from PKC to tight junction disruption. In MDCK I cells, p100/p120 dephosphorylation in response to PDB was followed closely by a drop in monolayer resistance. Rephosphorylation of p100 and p120 induced by inhibitors of PKC was followed by a re-establishment of resistance. If the phosphorylation state of p100/p120 alone is a key regulator of permeability, it would be expected that selective manipulation of phosphorylation should give predictable changes in permeability. The hyperphosphorylation of p100/p120 seen in response to the phosphatase inhibitors calyculin A and okadaic acid (Fig. 6), was not associated with a decrease in permeability. However, these pharmacological agents are unsuitable for analysis of physiological responses, since they also cause a disruption of the cytoskeleton (revealed by phalloidin labeling; result not shown), probably as a consequence of increased phosphorylation of many cellular proteins. Where p100/p120 dephosphorylation of many cellular proteins. Where p100/p120 dephosphorylation will require further investigation. However, our data clearly show is that the junctional proteins p100 and p120 are targets for a novel PKC signaling pathway.

Other potential roles for p100/p120 in signaling exist. There is a significant pool of p100/p120 that does not appear to interact with cadherins, which also undergoes dephosphorylation in response to PDB-stimulation. This p100/p120 pool may have some cellular function completely separate from any role in cell adhesion. PKC family members have many diverse roles in the cell: control of growth and proliferation, control of gene expression, regulation of cell morphology, and cell-matrix adhesion are just a few examples (see Refs. 69 and 70). p100/p120 dephosphorylation may be involved in any of these processes. Another member of the Armadillo family, β-catenin, has been shown to have roles in cell signaling pathways independent from its classical role in cell adhesion. For instance, β-catenin participates in the Wnt signaling pathway (see, for example, Refs. 71 and 72) and interacts with the adenomatus polyposis coli gene product (73, 74) as well as the transcription factors LEF-1 (75) and Tcf-3 (76). It is possible that p100 and p120 may also prove to have multiple functions. Determination of the biological significance of p100/p120 serine/threonine dephosphorylation will require further investigation. However, what our data clearly show is that the junctional proteins p100 and p120 are targets for a novel PKC signaling pathway.

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