Perspectives in Pharmacology

The Role of Protein Kinase C Isoforms in Modulating Injury and Repair of the Intestinal Barrier

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Received February 25, 2005; accepted June 30, 2005

ABSTRACT

Gastrointestinal cells express a diverse group of protein kinase C (PKC) isoforms that play critical roles in a number of cell functions, including intracellular signaling and barrier integrity. PKC isoforms expressed by gastrointestinal epithelial cells consist of three major PKC subfamilies: conventional isoforms (α, β1, β2, and γ), novel isoforms (δ, ε, ζ, η, and μ), and atypical isoforms (λ, σ, and θ). This review highlights recent discoveries, including our own, that some PKC isoforms in gastrointestinal epithelia monolayer cell culture are involved in injury to, whereas others are involved in protection of, intestinal barrier integrity. For example, certain PKC isoforms aggravate oxidative damage, whereas others protect against it. These findings suggest that the development of agents that selectively activate or inhibit specific PKC isoforms may lead to new therapeutic modalities for important gastrointestinal disorders such as cancer and inflammatory bowel disease.

This review focuses on protein kinase C (PKC) isoforms expressed in epithelial cells of the gastrointestinal tract, especially the intestine. PKC isoforms are involved in signal transduction in several vital intracellular pathways, including cell proliferation, differentiation, apoptosis, adhesion, membrane remodeling, migration, ion secretion, and barrier function. Excellent reviews have addressed PKC isoforms individually, but not as a group; nor has it been shown that specific PKC isoforms can be involved in damage, whereas others are involved in the protection of the same cells. The PKC isoforms expressed in intestinal tissue include at least 12 known isoenzymes that differ in their mechanism of activation, tissue expression, intracellular distribution, and substrate specificity. This suggests that each isoenzyme may have a unique role in signaling and cell function.

Barrier Function

The intestinal barrier is the largest interface between humans and their external environment (Farhadi et al., 2003).

As a filter with selective permeability, this interface allows absorption of required nutrients and excludes the penetration of harmful proinflammatory factors, including luminal antigens, microorganisms, and their products. The integrity of the intestinal barrier requires both healthy epithelial cells and a functionally normal paracellular pathway (tight junction). Disruption of the gastrointestinal barrier, on the other hand, permits penetration into the mucosa of normally excluded luminal substances and leads to the initiation or perpetuation of inflammatory processes (Keshavarzian et al., 2000).

An important structure essential for the integrity of both epithelial cells and paracellular transport is the cytoskeleton. This intracellular organelle has an intricate structure that extends throughout the cytosol and is involved in cell-cell contact through adherens junctions on the outer part of cells (Alvia, 1987). The cytoskeleton is also an essential structural component of paracellular pathways, which are controlled by tight junctions between epithelial cells. As might be expected, this pathway is a key regulator of intestinal permeability to macromolecules, such as bacterial byproducts, through dynamic changes in size that occur under various conditions.
physiological and pathological conditions (Madara, 1990). The cytoskeleton also serves as a pivotal element in maintaining normal structural and functional integrity of all eukaryotic cells, including gastrointestinal epithelium. Predictably, disruption of the cytoskeleton can severely limit cell function and disrupt the structural integrity of the intestinal barrier (Alvila, 1987).

Regulation of intestinal permeability involves a diverse array of intracellular mediators. For example, nitric oxide seems to be an important mediator for regulating intracellular functions and the microcirculation that nourishes cells (Alican and Kubes, 1996). A low level of nitric oxide, which is normally synthesized by constitutive nitric-oxide synthase, is important for maintenance of normal mucosal barrier function. However, overproduction of nitric oxide, which is carried out by inducible nitric-oxide synthase (iNOS), has been identified as a culprit in abnormal intestinal barrier function (Salzman et al., 1995). The mechanism by which nitric oxide overproduction induces intestinal barrier dysfunction is complex and multifactorial. It includes protein oxidation, nitration, S-nitrosylation, cGMP activation, and cellular energy depletion (Alican and Kubes, 1996).

In addition to nitric oxide, other reactive oxygen species (ROS) affect the intestinal barrier. Indeed, ROS has been implicated in the pathophysiology of aging and in numerous chronic disorders, including gastrointestinal disorders (Cross et al., 1987). Thus, although ROS functions as an intracellular messenger (e.g., nitric oxide) in normal physiologic signaling cascades triggered by growth factors or cytokines in all cells, including gastrointestinal epithelial cells, (Victor et al., 2000) under pathophysiological conditions, when intracellular levels of ROS increase and overwhelm the cell’s antioxidant capacity, damage occurs to cellular macromolecules such as lipids, proteins, and DNA. It is thus not surprising that oxidative stress can lead to intestinal barrier disruption. These dynamics also suggest that damaging and protective agents might modify intestinal permeability by, respectively, increasing or decreasing oxidative tissue damage.

Several other mediators of defense in epithelial cells protect them from noxious stimuli. One is epidermal growth factor (EGF). We and others have shown that EGF can prevent damage to gastrointestinal epithelium (Banan et al., 2002b) by oxidants or ethanol (Banan et al., 1999a). The exact mechanism underlying intracellular EGF signaling is not fully established, but it seems that this factor increases barrier and cytoskeletal integrity through changes in the activity of several protein kinases (Banan et al., 2001b). To understand the consequences of these changes in activity, it is helpful to discuss the intracellular roles of protein kinases.

### Protein Kinases

Protein kinases are key intracellular mediators of signal transduction pathways and are involved in various cell functions throughout the body, including membrane dynamics and permeability. This function provides them a pivotal role in barrier function in gastrointestinal epithelium and vascular endothelium. Protein kinases accomplish this regulation by their ability to phosphorylate proteins. Phosphorylation occurs at specific amino acids of proteins (e.g., serine, threonine, and tyrosine) and results in rapid changes in the activity of those proteins or enzymes. For instance, protein kinase A activation increases the ionic conductance of tight junctions without changing gastrointestinal barrier permeability for large molecules. Considering the whole family of the protein kinases, PKC is the one that has been studied more intensely in gastrointestinal epithelium and has been shown to be involved in several vital intracellular pathways, including barrier permeability (Karczewski and Groot, 2000). PKC is not a single entity but includes a subfamily of kinases that are serine- and threonine-specific, including at least 12 known isoenzymes. These can be classified into three subgroups based on differences in sequence homology and cofactor requirements (Davidson et al., 1994; Banan et al., 2001a). The first subgroup includes conventional or “classic” PKC isoforms and includes PKC-α, -β1, -β2, and -γ. Classic PKC isoforms require calcium, diacylglycerol, and phospholipids for their activation. The “novel” PKC isoenzymes include PKC-δ, -ε, -θ, -η, and -μ, which are similar to the conventional subgroup in that they still require diacylglycerol and phospholipid for activation but are calcium-independent. “Atypical” PKC isoforms are independent of both calcium and diacylglycerol and include PKC-α, -γ, and -ζ. Epithelial intestinal cells express at least 10 isoforms of PKC, including PKC-α, PKC-β1, PKC-β2, PKC-δ, PKC-ε, PKC-θ, PKC-η, PKC-ζ, PKC-α, and PKC-τ.

The distribution of PKC in the gastrointestinal tract is not limited to epithelial cells; PKC can be detected in gastrointestinal smooth muscle cells (Di Mari et al., 2003), cells of the cajal (Poole et al., 2004) and basal granulated cells (Kawakita et al., 1995). In this review, we only discuss the effects of PKC isoforms of intestinal epithelial cells. The differences in mode of activation, intracellular distribution, tissue expression, and substrate specificity of these isoforms suggest that there may be unique and nonredundant roles in gastrointestinal signal transduction for each isoenzyme. To begin to define these unique roles, we used monolayers of colon cancer intestinal cells as a model with which to study the integrity of intestinal cells and the intestinal barrier.

PKC isoforms in the gastrointestinal tract are involved in several vital intracellular pathways, including cell proliferation/cytostasis (Batlle et al., 1998; Verstovsek et al., 1998; Assert et al., 1999; Umar et al., 2000), cell differentiation (Abraham et al., 1998; Verstovsek et al., 1998; Frey et al., 2001), apoptosis (Chang and Tepperman, 2001; Frey et al., 2001), cell adhesion (Batlle et al., 1998; Holland et al., 2003), membrane remodeling (Song et al., 1999), epithelial migration (Andre et al., 1999), transepithelial permeability (Marano et al., 2001), secretion (Van den Berghe et al., 1992; Yoo et al., 2001), receptor and brush border enzyme expression (Murray et al., 2002), cytoskeletal modulation (Fasano et al., 1995; Banan et al., 2002a,b,c,d, 2003a,b, 2004a,b), tight junction modification (Clarke et al., 2000), and epithelial responses to inflammatory (Chang and Tepperman, 2001, 2003), cytotoxic (Tepperman et al., 1999; Chang and Tepperman, 2001), and carcinogenic (Pongracz et al., 1995; Murray et al., 1999, 2002; Perletti et al., 1999; Mullin et al., 2000) mediators. These multiple roles suggest that this group of enzymes is involved in the regulation of the health and damage of epithelial cells and the intestinal barrier. Thus, it is not surprising that these enzymes are modified by a wide variety of physical and physiological stimuli such as age (Balogh et al., 2000), diet (Murray et al., 2002), and hormones (Balogh et al., 2000). Nevertheless, the de-
A trend toward an increase in PKC-α is associated with the cessation of cell proliferation and increasing cell maturation. The total amount of PKC enzyme is associated with differentiation, which is even more complex. Studies show that in a decade ago that activation of PKC-α resulted in interactions between the mitotic phase in the crypt and the differentiation phase in the villous (Verstovsek et al., 1998). Other studies found that phorbol esters result in parallel activation of PKC-α, which is the key isoform responsible for early actin disassembly and basolateral membrane endocytosis induced by phorbol ester. Although Van den Berghe et al. (1992) noticed over a decade ago that activation of PKC-α is associated with ion secretion, the recent study by Song et al. (2002) showed that phorbol ester myristate acetate activated PKC-ε and PKC-α. Phorbol ester myristate acetate activation resulted in the redistribution of PKC-ε in basolateral membranes, which was associated with Cl secretion; the redistribution of PKC-α predominantly in apical membranes, which was associated with the modulation of transepithelial resistance (Song et al., 2002); increased epithelial permeability in an ex vivo model (Berin and Buell, 1995); and eventually intestinal inflammation in vivo models (Brown et al., 1999). Although Fasano et al. (1995) showed that zonula occludens toxin increases tight junction permeability and actin rearrangement through activation of PKC-α, recent studies did not confirm any significant changes in the composition and or localization of tight junction proteins, including occludin and zonula occludens-1 (Marano et al., 2001). This was also demonstrated by Holland et al. (2003), who showed that adherens junctions but not tight junctions are modulated by PKC-α. In addition, activation of PKC-α is associated with inactivation of E-cadherin, a key factor in the regulation of cell-cell contact, and might be the reason for multilayered cell growth (Batlle et al., 1998; Mullin et al., 2000). Song et al. (2002) showed that the effect on membrane remodeling is mainly mediated through stabilizing F-actin (the polymerized form; G-actin is the soluble form), and this effect opposes the effect of PKC-ε, which is the key isoform responsible for early actin disassembly and basolateral membrane endocytosis induced by phorbol ester. Although Van den Berghe et al. (1992) noticed over a decade ago that activation of PKC-α is associated with ion secretion, the recent study by Song et al. (2002) showed that phorbol ester myristate acetate activated PKC-ε and PKC-α. 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In addition, activation of PKC-α is associated with inactivation of E-cadherin, a key factor in the regulation of cell-cell contact, and might be the reason for multilayered cell growth (Batlle et al., 1998; Mullin et al., 2000). The role of PKC-α in epithelial cell proliferation and differentiation is even more complex. Studies show that increases in the total amount of PKC enzyme is associated with the cessation of cell proliferation and increasing cell maturation (Assert et al., 1999). Several investigators noted that there is a trend toward an increase in PKC-α as epithelial cells move from the mitotic phase in the crypt to the differentiating phase in the villous (Verstovsek et al., 1998). Other studies found that phorbol esters result in parallel activation of PKC-α and retardation of epithelial cell growth in a cell culture model (Batlle et al., 1998). However, measurements of PKC-α in colonic cancer tissue and the comparison of that with healthy mucosa has yielded conflicting results. Some studies show that the amount of PKC-α is decreased in polyp and cancer tissue (Perletti et al., 1999), whereas other studies did not find any differences (Pongracz et al., 1995; Banan et al., 2001a) or even found increases in the level of cytosolic PKC-α (Assert et al., 1999). Using a Caco-2 cell monolayer model, Abraham et al. (1998) showed PKC-α but not other PKCs are involved in proliferation/differentiation through a pathway involving p21waf1, a cyclin-dependent kinase inhibitor. Overall, it was concluded that PKC-α is involved in the process of cell maturation and proliferation and that its activation might result in multilayer cell proliferation and modulation of lateral cell-to-cell connections and transepithelial resistance.

**Conventional PKCs**

**PKC-α.** PKC-α has been associated with several cell functions. Mullin et al. (2000) and Marano et al. (2000) showed that phorbol ester tumor promoters, which are general PKC activators, cause immediate translocation of PKC-α from cytosolic- to membrane-associated compartments. This translocation is associated with paracellular leakiness and multilayered cell growth. This effect is attenuated by transfection of cells with dominant-negative-transfected cells for PKC-α (Mullin et al., 2000). Song et al. (2002) showed that the effect of PKC-α on membrane remodeling is mainly mediated through stabilizing F-actin (the polymerized form; G-actin is the soluble form), and this effect opposes the effect of PKC-ε, which is the key isoform responsible for early actin disassembly and basolateral membrane endocytosis induced by phorbol ester. Although Van den Berghe et al. (1992) noticed over a decade ago that activation of PKC-α is associated with ion secretion, the recent study by Song et al. (2002) showed that phorbol ester myristate acetate activated PKC-ε and PKC-α. Phorbol ester myristate acetate activation resulted in the redistribution of PKC-ε in basolateral membranes, which was associated with Cl secretion; the redistribution of PKC-α predominantly in apical membranes, which was associated with the modulation of transepithelial resistance (Song et al., 2002); increased epithelial permeability in an ex vivo model (Berin and Buell, 1995); and eventually intestinal inflammation in vivo models (Brown et al., 1999). Although Fasano et al. (1995) showed that zonula occludens toxin increases tight junction permeability and actin rearrangement through activation of PKC-α, recent studies did not confirm any significant changes in the composition and or localization of tight junction proteins, including occludin and zonula occludens-1 (Marano et al., 2001). This was also demonstrated by Holland et al. (2003), who showed that adherens junctions but not tight junctions are modulated by PKC-α. In addition, activation of PKC-α is associated with inactivation of E-cadherin, a key factor in the regulation of cell-cell contact, and might be the reason for multilayered cell growth (Batlle et al., 1998; Mullin et al., 2000).

The role of PKC-α in epithelial cell proliferation and differentiation is even more complex. Studies show that increases in the total amount of PKC enzyme is associated with the cessation of cell proliferation and increasing cell maturation (Assert et al., 1999). Several investigators noted that there is a trend toward an increase in PKC-α as epithelial cells move from the mitotic phase in the crypt to the differentiating phase in the villous (Verstovsek et al., 1998). Other studies found that phorbol esters result in parallel activation of PKC-α and retardation of epithelial cell growth in a cell culture model (Batlle et al., 1998). However, measurements of PKC-α in colonic cancer tissue and the comparison of that with healthy mucosa has yielded conflicting results. Some studies show that the amount of PKC-α is decreased in polyp and cancer tissue (Perletti et al., 1999), whereas other studies did not find any differences (Pongracz et al., 1995; Banan et al., 2001a) or even found increases in the level of cytosolic PKC-α (Assert et al., 1999). Using a Caco-2 cell monolayer model, Abraham et al. (1998) showed PKC-α but not other PKCs are involved in proliferation/differentiation through a pathway involving p21waf1, a cyclin-dependent kinase inhibitor. Overall, it was concluded that PKC-α is involved in the process of cell maturation and proliferation and that its activation might result in multilayer cell proliferation and modulation of lateral cell-to-cell connections and transepithelial resistance.

**PKC-β.** A role for the PKC-β isoform of PKC has mainly been explored in the field of barrier function (Karczewski and Groot, 2000). Using monolayers of human Caco-2 cells exposed to oxidants as a model of barrier disruption, we found that protection of barrier integrity is mediated by PKC-β, including stabilization of the microtubule and actin cytoskeletons through EGF or TGF-α signaling (Banan et al., 1999a, 2001b). (Fig. 1).

We also studied Caco-2 cells that were transfected to stably over- or underexpress PKC-β1. Using both transfection and pharmacological experiments, we showed that cell monolayers overexpressing PKC-β1 are protected from oxidant-induced injury through 1) enhanced architectural integrity of the microtubule cytoskeleton, 2) normalization of intracellular calcium levels by increases in calcium efflux, and 3) increases in the stable form of F-actin, which protects cellular architecture. This protection is associated with the redistribution of PKC-β1 from cytosolic pools to membrane- and cytoskeleton- bound pools (Fig. 1).

**Fig. 1.** Proposed schema for PKC-β1- and PKC-ε-dependent intestinal barrier protection.

![Fig. 1. Proposed schema for PKC-β1- and PKC-ε-dependent intestinal barrier protection.](image-url)
cytoskeletal-associated compartments. It is also associated with a novel biologic effect of PKC-β1 that we recently reported—stabilization of IκBα and prevention of NFκB activation. Antisense inhibition of PKC-β1 expression abolished EGF protection and was associated with unstable microtubule cytoarchitecture and barrier hyperpermeability (Banan et al., 2002b). Thus, PKC-β1 seems to be required for EGF-mediated protection of the gastrointestinal barrier. A role for PKC-β in cellular proliferation and differentiation is more speculative. Some studies show that the β isoform of PKC is detectable in higher levels in highly proliferative or tumor tissue. Davidson et al. (1994) found that PKC-β mRNA and cytosolic PKC-β are seen in higher amounts in colonic adenomatous polyps. Later, they showed that increases in PKC-β2 mRNA in fecal tests correlate with the incidence of colon cancer (Davidson et al., 1998). In contrast, Pongracz et al. (1995) found a lower amount of PKC-β in cancer tissue than in controls (Pongracz et al., 1995). This finding was later reproduced by Assert et al. (1999), who found that PKC-β mRNA levels were decreased in colon cancer tissues. Assert et al. (1999) also showed that whereas the level of cytosolic PKC-β1 is not different in adenomatous polyps, membrane-associated PKC-β2 is significantly lowered in these polyps (Assert et al., 1999). This finding is compatible with studies done by Verstovsek et al. (1998), who showed that the level of PKC-β2 is low in proliferating crypt cells and increases as cells mature during their migration upward toward the villi. This controversy remained unresolved until Murray et al. (1999) showed that in transgenic mice that overexpress PKC-β2 in intestinal epithelium, colonic proliferation is promoted and signaling of the adenomatous polyposis coli gene is activated. Later, this group showed that the mechanism of promotion of colon carcinogenesis of PKC-β2 is through overexpression of cyclooxygenase-2 and eventually repression of TGF-β type 2 receptors, which have growth inhibitory properties when stimulated by TGF-β (Murray et al., 2002). They also suggested a molecular mechanism through which nonsteroidal anti-inflammatory drugs and omega-3 fatty acids suppress colon carcinogenesis. In this model, omega-3 fatty acids and nonsteroidal anti-inflammatory drugs restore the responsiveness of this PKC-β2 signaling axis (Murray et al., 2002).

PKC-γ. This is one of the least explored isoforms of the PK family. It is considered a conventional PKC, but knowledge about it is limited. Andre et al. (1999) showed that PKC-γ is involved in insulin-like growth factor-induced colonic epithelial cell migration and thus could be important in tissue repair (Andre et al., 1999). This isoform could not be detected in most colonic neoplasms and could not be detected in exfoliated colonocytes (Banan et al., 2001a).

Novel PKCs

PKC-δ. This isoenzyme is one of the calcium-independent PKC isoforms. However, like classic PKC isoforms, it requires both diacylglycerol and phospholipid for activation. Several recent studies have addressed the role of PKC-δ in various cellular processes from cytotoxicity and cell barrier protection to proliferation and differentiation. It has been postulated that the cytotoxicity and apoptosis induced by tumor necrosis factor (TNF) during inflammatory processes is mediated through PKC-δ signaling. Chang and Tepperman (2001) reported that TNF-α induced translocation of PKC-α, PKC-δ, and PKC-ε from cytosol to membrane, and the cytotoxicity and apoptosis induced by TNF-α were reduced using inhibitors of PKC-δ and PKC-ε. We showed, using a monolayer model, that exposure to oxidants causes PKC-δ activation and translocation to particulate fractions, which coincided with disruption of barrier integrity (Banan et al., 2002a). Using transfection, we showed that overexpression of PKC-δ results in increased PKC-δ in the particulate fraction (active form) and in cellular damage even without exposure of cells to oxidants. In addition, dominant-negative-transfected cells showed 98% inhibition of native PKC-δ enzyme activity and protected the cell against oxidant-induced injury (Banan et al., 2002a). We pointed out that the mechanism through which PKC-δ damages cells is tightly associated with iNOS up-regulation, which is a common pathway through which stimuli damage cells (Banan et al., 2003a). Chang and Tepperman (2003) recently reported that the mechanism of injury from PKC-δ and PKC-ε activation involves degradation of IκBα and activation of NFκB. These observations suggest a pathway through which PKC-δ and PKC-ε exert their cytopathic effects (Fig. 2).

PKC-δ has also been implicated in cellular proliferation and differentiation. In early reports, Davidson et al. (1994) showed that colonic adenocarcinoma tissue has higher amounts of PKC-δ, particularly in the membrane-associated fraction. Findings of Assert et al. (1999) did not agree with the finding of Davidson et al. (1994), failing to show increases in PKC-δ mRNA in tumor samples. This latter finding was confirmed by Perletti et al. (1999) in an in vitro model of colon carcinogenesis, which showed that PKC-δ is significantly

![Fig. 2. Proposed schema of PKC-δ- and PKC-ε-dependent intestinal barrier disruption.](image-url)
decreased in rat epithelial cells with a neoplastic phenotype, and overexpression of PKC-δ could reverse the suppression of cell growth in these cells. In addition, down-regulation of PKC-δ can promote neoplastic transformation (Perletti et al., 1999). Frey et al. (2001) showed that bistratene A, which is a potent agent that induces cytostasis and cell differentiation, modulates its effect, partly through activation of PKC-δ. The other known role for PKC-δ is in conjunction with PKC-γ in colonoctye restitution induced by insulin-like growth factor (Andre et al., 1999) and the response of intestinal epithelial cells to leukotriene D4, an inflammatory mediator (Masoumi et al., 2002).

**PKC-ε.** Nitric oxide-induced cell injury is associated with an increased level of PKC-ε, and this increase is accompanied by redistribution of PKC-ε from the cytosolic compartment to the membrane (active) fraction (Tepperman et al., 1999). Through a series of pharmacological experiments using a monolayer model, Yoo et al. (2001) showed that PKC-ε may inhibit hypoxia-induced secretory responses in epithelial cells. This finding was later reproduced by Saksena et al. (2002), who showed that EGF inhibited basolateral Cl− secretion through activation of PKC-ε. Later studies suggested that PKC-ε has an inhibitory role in TNF-induced barrier dysfunction (Yoo et al., 2001). However, this view was seriously challenged by another study that showed that PKC-ε mediates cytotoxicity and apoptosis in cells exposed to TNF-α (Chang and Tepperman, 2001). On the other hand, Song et al. (1999, 2002) showed that PKC-ε may have a role in the stimulation of basolateral membrane endocytosis and remodeling, and this effect opposes the PKC-α effect on membrane stabilization. Yoo et al. (2003) showed that bryostatin-1-induced changes in barrier function were attenuated by the conventional and novel PKC inhibitor Gö-6850 (but not by the conventional PKC inhibitor Gö-6976 or the PKC inhibitor rottlerin), implicating a novel isoenzyme, likely PKC-ε.

A role for PKC-ε in the proliferation and differentiation of epithelial cells has not yet been found. This isoform modulates mucin gene expression in epithelial cells (Hong et al., 1999). In addition, Pongracz et al. (1995) showed that the level of PKC-ε in tumor tissue is extremely low. However, Perletti et al. (1999) failed to show a significant role for PKC-ε in a neoplastic phenotype rat model.

**PKC-θ.** In a recent study, we showed that the PKC-θ isoform is a required part of cytoskeletal assembly and barrier function in monolayers (Banan et al., 2004b). The molecular events underlying this novel biological effect of PKC-θ involves changes in phosphorylation and/or assembly of the cytoskeleton. The ability to alter cytoskeletal and barrier dynamics is a unique function not previously attributed to PKC-θ.

**Atypical PKCs**

**PKC-ζ.** This atypical PKC isoform is independent of both calcium and diacylglycerol, and its functional properties have recently attracted the attention of many scientists in the field of epithelial biology. We reported the initial study of the involvement of PKC-ζ in modulating intestinal barrier function. In our early study, we reported that EGF activated PKC-α, PKC-β1, and PKC-ζ, and this activation resulted in the redistribution of these enzymes from cytosolic to membrane compartments, which resulted in protection of the intestinal barrier against oxidant-induced damage (Banan et al., 2001b). Later, we used a transfected Caco-2 cell monolayer model and were able to stably over- and underexpress PKC-ζ in these cells. We showed that PKC-ζ is an essential part of EGF-induced protection of the intestinal barrier and that cells with overexpressed PKC-ζ are protected against oxidants even in the absence of EGF. Inhibition of expression of PKC-ζ resulted in attenuation of EGF protection against oxidants and loss of barrier integrity (Banan et al., 2002c). The protective mechanism involved enhanced the stability of the microtubule and cytoskeleton against oxidative stress and was mediated by down-regulation of iNOS (Banan et al., 2002d) and inhibition of the activation of NFκB through the suppression of phosphorylation and enhancement of stabilisation of IκB (Banan et al., 2003b) (Fig. 1).

A role for PKC-ζ in cell proliferation and differentiation is still not fully understood. Pongracz et al. (1995) did not find any difference in the amount of PKC-ζ in tumor tissue compared with normal colon. However, Davidson et al. (1998) used the level of mRNA of this PKC isoform as a marker for neoplastic activity. In their study, Davidson et al. noticed a lower level of PKC-ζ mRNA in fecal samples in a colon carcinoma model. In particular, they found that the ratio of PKC-β2/PKC-ζ mRNA was useful as a noninvasive marker for the surveillance of colon cancer (Davidson et al., 1998). Verstovsek et al. (1998) also showed a relatively low level of PKC-ζ, particularly in the cytosolic compartment in proliferating cells of the crypt base. As these cells cease proliferation and start differentiation, they move toward the villi, a process that coincides with increases in the level of PKC-ζ, particularly in the membrane- and cytoskeletal-associated form. Umar et al. (2000) successfully fractionated PKC-ζ into three cellular fractions, PKC-ζ holoenzyme, catalytic subunit, and membrane-bound fragment, and noticed that all three types are associated with enhanced mitosis in colonocytes in a transmissible murine colon hyperplasia.

**PKC-λ.** This atypical PKC isoform has the ability to induce oxidant-like injury, including cytoskeletal depolymerization and instability. In fact, in our recent study, we showed that oxidant induces disruption of epithelial barrier integrity, in large part through activation of the PKC-λ isoform (Banan et al., 2004a). The cells that did not express endogenous PKC-λ because of having been transfected with a dominant-negative gene were protected against all measures of oxidant-induced disruption. The ability to cause oxidative tissue damage is a novel mechanism not previously attributed to the atypical subfamily of PKC isoforms.

**Future Therapeutic Direction and Conclusions**

Although PKC isoforms modulate almost all vital functions of cells, our knowledge of their mechanisms is limited. This is due to several experimental impediments in this field. The most important is the lack of specific activators and inhibitors of PKC isoforms, which would be important pharmacologic tools in the investigation of these PKC isoforms. In addition, the very fact that PKC isoenzymes are involved in so many biological functions of cells makes it hard to experimentally isolate individual intracellular pathways in cells without interfering with parallel pathways. Moreover, compartment-specific PKC isoforms (e.g., membrane-associated, nuclear, or cytosolic) or subisoforms (e.g., PKC-β1 and PKC-β2) might have different or even opposite functions, and this
makes interpreting experimental outcomes difficult. In addition, most PKC studies use an intestinal monolayer model made of colon cancer cells, which might be a potential source of conflicting results. These malignant cells may behave differently compared with normal intestinal epithelial cells in culture, in vivo gastrointestinal epithelia, or even to other cells in the body. Another issue is deciding what protection and/or damage mean. For example, in an in vitro model, a protective PKC isoform may protect the cell against damage by noxious stimuli (e.g., oxidants), but in an in vivo model, this same PKC isoform may protect cancer cells against immune system attack and thus promote cancer cell survival. Should we consider this PKC as protective?

Despite these difficulties in investigating the roles of different PKC isoforms in biological models, progress has been made in investigating PKC status in various gastrointestinal diseases. For example, PKC activators and inhibitors have been used with some success in animal models of colitis. As mentioned earlier, generalized PKC activation induces colitis in animal models, whereas generalized PKC inhibition protects against the development of colitis. It seems that the barrier integrity of gastrointestinal epithelium coincides with a homeostatic balance among various PKC isoforms, and imbalances in this homeostasis can result in either barrier dysfunction or barrier overprotection. PKC-α, PKC-δ, PKC-ε, and PKC-λ seem to be involved in epithelial barrier dysfunction, whereas PKC-β1, PKC-θ, and PKC-ζ protect barrier integrity. PKC-α, PKC-δ2, PKC-δ, and PKC-ζ are involved in cell proliferation and possibly, therefore, carcinogenesis. Increasing our knowledge of the basic functions of the various isoforms of PKC will make it possible to develop specific activators and inhibitors of individual PKC isoenzymes, and this should lead to our ability to dissect individual PKC functions. It should also lead to new and improved strategies for the management of gastrointestinal disorders. As we enter this largely unexplored area, several basic questions will have to be answered. Why is there so much diversity in the cellular functions that PKC isoenzymes mediate? Why do many PKC isoforms protect cells and barrier function, whereas other PKC isoforms cause damage? Why should cells harbor damaging factors? Could this mechanism be useful for controlling cell differentiation and proliferation? Or could this mechanism be part of programmed cell death (apoptosis)? Our ability to answer these questions is at present limited. Fortunately, however, new techniques and agents are continually being developed in pharmacology and molecular biology studies and hold promise for helping to answer the above questions. This should lead to new insights into PKC-mediated intracellular mechanisms and may open new windows for the treatment of a variety of disorders.

References


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