Protein Kinase C Mediates Lipopolysaccharide- and Phorbol-Induced Nitric-Oxide Synthase Activity and Cellular Injury in the Rat Colon

BARRY L. TEPPERMAN, QING CHANG, and BRIAN D. SOPER
Department of Physiology, University of Western Ontario, London, Ontario, Canada
Accepted for publication August 22, 2000 This paper is available online at http://www.jpet.org

ABSTRACT
The role of protein kinase C (PKC) in lipopolysaccharide (LPS)- and phorbol ester-induced changes in rat colonic cellular integrity and Ca$^{2+}$-independent inducible nitric-oxide synthase (iNOS) activity was investigated. LPS treatment (3 mg kg$^{-1}$ i.p.) increased colonic cellular PKC activity within 1 h after administration. The percentage of nonviable cells and iNOS activity in response to LPS were reduced by pretreatment with the selective PKC antagonist GF 109203X (25 ng kg$^{-1}$ i.v.). Pretreatment with the selective iNOS inhibitor 1400W reduced the extent of cellular injury and iNOS activity but did not affect the increase in PKC activity. Reduction of circulating neutrophils with anti-neutrophil serum reduced cell damage as well as the increases in PKC and iNOS activities in response to LPS. Intracolonic administration of the phorbol ester phorbol-12-myristate-13-acetate (PMA; 3 mg kg$^{-1}$) increased colonic cellular PKC activity within 2 h after instillation. Cellular iNOS activity did not increase until 6 h after PMA administration. The colonic responses to PMA were eliminated by GF 109203X. The selective iNOS inhibitor 1400W reduced the increase in cell injury but did not affect the PKC activation in response to PMA. LPS treatment also increased in the proteins for PKC-α, PKC-δ, PKC-ε, and PKC-ζ. PMA treatment resulted in PKC-δ and PKC-ε translocation from cytosol to membrane. These data suggest that PKC mediates iNOS activation and subsequent colonic cell injury in response to LPS administration. The δ- and ε-isozymes appear to be most closely associated with these responses.

Administration of bacterial endotoxin via lipopolysaccharide (LPS) treatment to rats has been associated with changes in intestinal vascular permeability and with cytotoxic actions on small and large intestinal epithelial cells (Bough-aston-Smith et al., 1993; Tepperman et al., 1994). Furthermore, LPS administration has also been shown to induce Ca$^{2+}$-independent nitric-oxide synthase (NOS) activity. The large amounts of nitric oxide (NO) elaborated as a result of this enhanced enzyme activity have been shown to mediate the injury associated with LPS treatment and inhibition of this induction can ameliorate the extent of cellular damage (Tepperman et al., 1994).

The mechanism whereby the LPS signal is transduced into the enhanced inducible NOS (iNOS) activity in intestinal epithelial cells is unknown. However, it has been shown that protein kinase C (PKC) is an important mediator for LPS-induced NOS activity as well as dysfunctional changes in the contractility of rat vascular tissue (McKenna et al., 1994). Similarly, it has been shown in rat macrophages that direct activation of PKC can induce iNOS activity (Hortelano et al., 1993).

Protein kinase C consists of a family of at least 12 serine-threonine protein kinases that have been implicated in many cellular signaling pathways (Nishizuka, 1992). In addition to its signal transduction role, PKC has also been associated with tissue injury. PKC activation is associated with inflammation of a number of tissues, including the colon (Gupta et al., 1988; Sakanoue et al., 1992). Furthermore, direct activation of PKC via intraluminal instillation of phorbol ester has been shown to induce ileal and colonic inflammation in experimental animals (Fretland et al., 1990; Buell and Berin, 1994; Berin and Buell, 1995; Overdahl et al., 1995). Recently, this laboratory has demonstrated that trinitrobenzene sulfonic acid-induced colonic mucosal injury is mediated by increases in PKC activity (Brown et al., 1999). Furthermore, activation of PKC has also been shown to compromise the viability of a variety of cell types in vitro and PKC activity has been shown to be elevated in cells in response to a number of inflammatory challenges (Kuruvilla et al., 1993; Koong et al., 1994; Jan et al., 1997; Jones et al., 1997).

Therefore, in the present study we have examined the

ABBREVIATIONS: LPS, lipopolysaccharide; NO, nitric oxide; iNOS, inducible nitric-oxide synthase; PKC, protein kinase C; PMA, phorbol-12-myristate 13-acetate; ANS, anti-neutrophil serum; MPO, myeloperoxidase; ECL, enhanced chemiluminescence.
possibility that LPS administration activates the Ca\textsuperscript{2+}-independent iNOS in colonic epithelial cells via an increase in PKC activity and by this route mediates the observed decreases in cellular integrity. We have also examined the effect of direct activation of PKC in colonic epithelium via intraluminal administration of phosphorib ester on promoting cellular iNOS as well as changes in integrity.

**Experimental Procedures**

**Isolation of Colonic Epithelial Cells**

Nonfasted male Sprague-Dawley rats (250–300 g) were sacrificed by cervical dislocation, and colonic epithelial cells were isolated from the colonic mucosa. Briefly, the colon was excised, everted, rinsed in ice-cold saline, and distended with Dulbecco’s phosphate buffer (pH 7.2). The colonic segment was incubated for 15 min at 37°C in 50 ml of Dulbecco’s in a water bath that shook at 50 oscillations min\textsuperscript{-1} to remove dead cells. The distended colonic sacs were then suspended in 25 ml of RPMI 1640 medium (1 mg ml\textsuperscript{-1} containing 1 mM EDTA and 1 mg ml\textsuperscript{-1} hyaluronidase (Sigma, St. Louis, MO) for 30 min at 37°C. Cells were removed by vigorous shaking and the cells collected were centrifuged at 2000g for 2 min. The cells were washed twice in RPMI 1640 buffer and filtered once more before their use in the experiments.

**Treatments**

In some experiments, the rats were treated with bacterial LPS from *Escherichia coli* (serotype 0111:B4; Sigma; 3 mg kg\textsuperscript{-1} i.v.) Animals were killed and the colons removed for cell harvest at intervals of 1 to 6 h after LPS treatment. In further experiments, groups of rats were treated with the protein kinase C activator phorbol-12-myristate 13-acetate (PMA; 3 mg kg\textsuperscript{-1} intracolonically; Biomol, Plymouth Meeting, PA). The PMA was instilled in a volume of 0.5 ml or less. In these experiments, animals were killed and the colons excised for cell isolation 1 to 6 h after instillation of the PMA.

In animals in these groups of studies were also treated with the following agents: the highly selective PKC antagonist GF 109203X (Toulec et al., 1991) administered in vivo at a concentration of 25 ng kg\textsuperscript{-1} i.v., and the specific inhibitor of iNOS 1400W (N-(3-aminomethyl)benzyl) acetamide, 5 mg kg\textsuperscript{-1} s.c.; Alexis Biochemicals, San Diego, CA) (Garvey et al., 1997). These agents used at the doses described above have been shown to effectively inhibit PKC and iNOS activities, respectively, in vivo (Laszlo and Whittle, 1997; Brown et al., 1999). Both agents were given 15 min before either LPS or PMA treatments and animals killed 2 h after LPS administration and 4 h after PMA treatment. Furthermore, in the LPS studies, groups of animals were pretreated with anti-neutrophil serum (ANS; Accurate Chemical and Scientific Corporation, Westbury, NY; 10 ml of antiserum i.p. 2 h before LPS), which at this dose and with the same treatment regime has previously been shown to reduce the number of circulating neutrophils to less than 5% of control numbers (Brown et al., 1998). In these experiments rats were killed, the colons excised, and cells harvested 2 h after LPS treatment as described above. In a final group of studies, rats were treated with ANS as described above and 2 h later the animals were killed, the colons excised, and cells harvested. Control animals were treated with a similar volume of normal rabbit serum. The resultant cellular suspensions were then treated in vitro with the PKC activator PMA in the concentration range 0.1 to 10 \mu M. Cells were incubated with PMA for 20 min at 37°C after which time cells were examined for viability, PKC activity, and iNOS activation.

**Trypan Blue Dye Uptake**

In all experiments an aliquot of cells was examined for viability as determined by Trypan blue dye exclusion (0.5% Trypan blue in phosphate-buffered saline), which has previously been shown to be a reliable index of gastrointestinal epithelial cell injury (Tepperman et al., 1991). Cells were counted in a randomized manner by a naive observer using a hemocytometer and the number of nonviable cells was determined by light microscopy (200× magnification) by counting those cells that failed to exclude the dye.

**Measurement of Myeloperoxidase (MPO) Activity**

MPO levels were measured to provide an index of polymorphonuclear leukocyte infiltration. MPO activity was determined as described by Wallace (1987). Briefly, samples of cells (4 x 10\textsuperscript{6} cells) were resuspended in 50 mM phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (1 ml; pH 6.0). Samples were frozen in liquid nitrogen and thawed. This procedure was repeated twice more. Samples were then centrifuged at 40,000g for 15 min at 4°C. MPO activity in the supernatant was determined by adding 100 \mu l of the supernatant to 2.9 ml of 50 mM phosphate buffer containing 0.167 mg ml\textsuperscript{-1} o-dianisidine hydrochloride (Sigma) and 0.0005% w/v hydrogen peroxide. The change in absorbance at 460 nm over a 3-min period was measured. MPO activity is presented as moles of hydrogen peroxide converted to water per 4 x 10\textsuperscript{6} cells.

**Measurement of Protein Kinase C Activity**

Cells were centrifuged at 2000g for 10 min (4°C) and then were resuspended in 50 mM Tris-HCl buffer (pH 7.4) containing EDTA (5 mM), EGTA (10 mM), phenylmethylsulfonyl fluoride (50 \mu g ml\textsuperscript{-1}), benzamide (10 mM), soybean trypsin inhibitor (10 \mu g ml\textsuperscript{-1}), leupeptin (10 \mu g ml\textsuperscript{-1}), aprotinin (10 \mu g ml\textsuperscript{-1}), \beta-mercaptoethanol (0.3% w/v), and okadaic acid (10 nM). The cells were lysed by sonication (10 s). A 25-\mu l aliquot of the sonicate was removed for determination of PKC activity using a commercially available kit (Amersham, Burlingon, Ontario, Canada), which measures the transfer of [γ-\textsuperscript{32}P]ATP to a peptide specific for PKC. Results are expressed as picomoles per minute per 10\textsuperscript{6} cells.

**Measurement of Protein Kinase C Content**

Materials. Affinity-purified rabbit polyclonal antibodies to the α-, β-, δ-, ε-, and ζ-isoforms of protein kinase C were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The secondary antibody was a goat anti-rabbit antibody conjugated to horseradish peroxidase purchased from Amersham (Arlington Heights, IL). Rainbow electrophoresis molecular weight marker, the enhanced chemiluminescence (ECL) kit, Hybond ECL nitrocellulose membrane, and Hyperfilm ECL were also purchased from Amersham.

**Preparation of Cytosolic and Particulate Fractions.** Some cells were resuspended in buffer and sonicated for 15 s on ice. The buffer consisted of 50 mM Tris-HCl (pH 7.5); 0.25 M sucrose; 2 mM EDTA; 1 mM EGTA; 25 \mu g ml\textsuperscript{-1} each of aprotinin, leupeptin, and pepstatin; 1 \mu g ml\textsuperscript{-1} soybean trypsin inhibitor; 50 \mu g ml\textsuperscript{-1} phenylmethylsulfonyl fluoride; and 10 mM β-mercaptoethanol. The samples were centrifuged at 100,000g for 60 min. The supernatant was taken as the cytosolic fraction. The pellet was resuspended in the buffer described above to which was added 10% Triton X-100 and extracted at 4°C for 1 h before centrifugation again under the same conditions. The whole cellular sample was extracted by the same homogenization buffer containing Triton X-100 and centrifuged at 25,000g for 20 min. The protein concentration of each sample was subsequently determined.

**Immunoblotting of Cellular Samples.** Each sample of 10 to 15 \mu g of protein was boiled for 10 min in an equal volume of sample buffer (125 mM Tris-HCl, pH 6.8; 20% glycerol; and 10% mercaptoethanol) before subjecting to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoresis, the gel was soaked for 30 min in transfer buffer and electroblotted onto nitrocellulose membrane using Mini-Trans blot. A corresponding gel with the same loading samples and broad-range standard protein markers (Bio-Rad, Hercules, CA) were stained with Coomassie Brilliant blue R-250. The bands appearing on the whole gel were scanned to dem-
onstrate standards of equal loading. Furthermore, after electrophoresis, the gel was cut according to the position of the standard protein marker. The region around the PKC protein molecular weight was cut for further blotting analysis. The lower portions were stained to demonstrate the bands on the gel as a marker of equal loading.

The membranes were blocked for 1 h with 10% nonfat dry milk in phosphate-buffered saline [80 mM Na$_2$PO$_4$, 20 mM NaH$_2$PO$_4$, 10 mM NaCl, and 0.05% Tween 20 (pH 7.5)]. The blots were then incubated for 3 h with specific protein kinase C-α antibody (1:1000), protein kinase C-ε antibody (1:800), or protein kinase C-γ antibody (1:800) at room temperature. The individual blocking peptides were incubated with each specific antibody to confirm the specific binding. After washes with phosphate-buffered saline (three times for 10 min), a 1:5000 dilution of horseradish peroxidase-linked secondary antibody was added for 2 h at room temperature. The ECL kit was used to visualize the immunoreactive bands according to the manufacturer’s protocol. The density of the immunoreactive bands on the autoradiogram was quantified by measurement of the absolute integrated optical density, which estimates the volume of the band in the lane profile as calculated by Image Master VDS software (Pharmacia Biotech, Uppsala, Sweden).

**Measurement of NOS Activity**

Cellular NOS activity was assessed by determining the formation of radiolabeled citrulline from the substrate $^{14}$C-arginine as described previously for colonic mucosal cells (Tepperman et al., 1994). Briefly, enzyme activity was released from cells into the buffer by freezing in liquid nitrogen and then thawing at 37°C three times. The broken cell fractions were then centrifuged at 10,000 g for 20 min. The supernatant was removed for estimation of the radiolabeled products added to bind arginine. The resin was allowed to settle and the supernatant was removed for estimation of the radiolabeled products added to bind arginine.

**Statistics**

Data are shown as means ± S.E.M. of six to eight experiments each done in duplicate. Statistical significance was assessed by the $t$ test for paired data or one-way analysis of variance and Duncan’s multiple range test where $P < .05$ was taken as significant.

**Results**

**Effect of Lipopolysaccharide.** Cells isolated from the rat colonic mucosa by the techniques used here were identified by light microscopy as being 90 to 95% epithelial cells. Lipopolysaccharide treatment resulted in an increase in the extent of cell injury as assessed by Trypan blue dye uptake (Fig. 1A). A significant increase in the extent of cell damage was not observed until 2 h after LPS administration. The peak extent of cell damage was seen 4 h after LPS.

Cellular protein kinase C activity was significantly elevated within the 1st h after LPS treatment. Levels declined over the next 3 h but still remained significantly elevated when compared with control levels at 6 h after LPS injection (Fig. 1B). In contrast Ca$^{2+}$-independent inducible nitric-oxide synthase activity was increased significantly by 2 h after LPS treatment and reached peak and stable levels of enzyme activity at 4 and 6 h after LPS (Fig. 1C).

The effect of LPS on cell injury was significantly reduced by pretreating rats with the selective PKC inhibitor GF 109203X (Fig. 2A). This concentration of the PKC antagonist also inhibited the LPS-mediated stimulation of cellular PKC activity seen 2 h after LPS administration (Fig. 2B). Furthermore, GF 109203X also significantly reduced the small increase in iNOS activity observed 2 h after LPS administration (data not shown).

Similarly, administration of the selective iNOS inhibitor 1400W significantly reduced the extent of cell damage as well as the increase in iNOS activity in response to LPS. However, there was no significant effect of 1400W on the cellular PKC response to LPS administration (Fig. 2, A and B).

Anti-neutrophil treatment significantly reduced the increase in myeloperoxidase in response to LPS administration as well as the increases in the activities in PKC and iNOS (Fig. 3, B–D). Furthermore, ANS pretreatment also significantly reduced Trypan blue dye uptake, indicating a decrease in the extent of cellular injury in response to LPS administration (Fig. 3A).
LPS treatment resulted in an increase in the protein levels for each of the PKC isoforms examined. Thus, PKC-α and -ζ were each increased within the 1st h after LPS administration (Fig. 4) and remained increased over the 6-h period observed after LPS treatment. PKC-δ and PKC-ε proteins appeared to be down-regulated from 1 to 2 h after LPS and returned to normal levels by 4 h but were increased over control at 4 and 6 h after treatment (Fig. 4). PKC-ε protein

Fig. 2. Effect of pretreatment (15 min) with either the selective PKC inhibitor GF 109203X (25 ng kg⁻¹ i.v.) or the selective iNOS inhibitor 1400W (5 mg kg⁻¹ s.c.) on the extent of cellular injury as assessed by Trypan blue dye uptake (A), PKC activity (B), and Ca²⁺-independent NOS activity (C) in response to LPS administration. Animals were killed 2 h after LPS administration. Values are displayed as means ± S.E.M. of six to eight independent experiments. Asterisks (*) indicate significant reductions from the LPS alone data as determined by Duncan’s multiple range test.
levels were increased at 2 h and remained elevated over the 6-h period after treatment.  

Effect of Phorbol Myristate Acetate. Phorbol myristate acetate treatment of cells in vitro resulted in a dose-dependent increase in the extent of cell damage (Fig. 5A) as well as an increase in PKC activity in cells harvested from control rats (Fig. 5B). PMA treatment resulted in a significant increase in iNOS activity in response to 10 μM PMA (Fig. 5C). Repetition of this experiment in cells harvested from animals made neutropenic by ANS treatment resulted in significant reductions in the extent of cell injury, and PKC and iNOS activities in response to PMA treatment (Fig. 5) although, especially at the highest concentration of phorbol ester used, the values were significantly greater that those observed for the respective control samples.

Intracolonic administration of the phorbol ester PMA resulted in an increase in the extent of cell injury when compared with vehicle control (20% ethanol) responses (Fig. 6A). The increase in Trypan blue uptake was seen within the 1st h after PMA treatment and increased slowly over the next 5 h after PMA treatment.

PKC activity was also increased significantly over vehicle control responses by 1 h after treatment and reached peak increases 2 h after PMA. Thereafter, PKC activity progressively declined over the remaining 4 h (Fig. 6B). In contrast, iNOS activity was significantly increased over vehicle control levels at 4 and 6 h after PMA (Fig. 6C).

The effect of PMA on cellular integrity and PKC activity examined 4 h after administration of the phorbol ester was significantly reduced by the prior administration of the PKC inhibitor GF 109203X (Fig. 7, A and B). Pretreatment with the iNOS inhibitor 1400W reduced the degree of cell injury but did not significantly inhibit PKC activity in response to PMA treatment.

The effect of PMA on the translocation of PKC isoforms is shown in Fig. 8. PMA treatment resulted in the translocation from cytosol to membrane of PKC-δ and PKC-ε but not for PKC-α. PKC-ζ was not activated by PMA treatment.

Discussion

The results of the present study indicate that administration of bacterial endotoxin LPS to experimental animals resulted in an increase in the extent of cell injury as well as an
increase in inducible NOS activity in epithelial cells harvested from the colonic mucosa. This confirms previous findings that LPS treatment would activate iNOS and the resultant NO thus liberated could account for the increase in colonic cellular damage (Tepperman et al., 1994). Furthermore, the present data indicate that LPS treatment results in an increase in PKC activity in the cells isolated from the colonic mucosa. The increase in PKC occurs within the 1st h after LPS treatment, whereas the increase in iNOS activity was not observed until 4 h after LPS injection, a time that corresponded to the increase in the extent of cell injury. This temporal relationship suggests that the increase in PKC may mediate the cellular injury via activation of iNOS. This suggestion is further supported by the finding that the effect of LPS on iNOS activation could be inhibited by pretreating animals with the selective PKC inhibitor GF 109203X. On the other hand, the selective iNOS inhibitor 1400W did not affect cellular PKC activity. These findings support previous studies, which have demonstrated that LPS administration would increase iNOS activity via PKC activation in a variety of cell types, including cardiac cells (McKenna et al., 1995), aortic smooth muscle cells (Paul et al., 1997), macrophages (Shapira et al., 1997), and microglial cells (Fiebich et al., 1998).

The present study also demonstrated that this increase in PKC activity in vivo as well as the increase in cell injury in response to LPS was dependent, at least in part, upon neutrophil infiltration. These data could suggest that the neutrophil is the source of the PKC activity determined in this study, and its reduction after anti-neutrophil serum treatment accounts for the reduction in both iNOS activity and the extent of cell injury in response to LPS. These data do not exclude the possibility that the neutrophil may also contribute directly to the increase in iNOS activity seen after LPS or...
PMA activation. Alternatively, these data may also indicate that the infiltrating cells that secrete a wide variety of inflammatory mediators such as tumor necrosis factor-α and interleukin-1 (Nathan, 1987) may stimulate the colonic epithelial cells to increase PKC activity and subsequently, iNOS activity. Previous studies have demonstrated that such cytokines can increase PKC activity in some epithelial cells (Wyatt et al., 1997; Prasanna et al., 1998; Fischer et al., 1999). This possibility is supported to some extent by our in vitro findings in which elimination of neutrophils by ANS treatment resulted in a reduction in the extent of cell injury as well as PKC and iNOS activities in response to direct stimulation of the cells with the PKC activator PMA. The remaining epithelial cells were able to respond to the phorbol ester but only at the highest concentration used here. Therefore, in the present study it is likely that activation of PKC and iNOS occur predominantly within the neutrophils.

We have also examined the effects of direct activation of PKC via the intraluminal administration of the phorbol ester PMA. In the present study, we have determined that administration of PMA by this route resulted in a rapid increase in the extent of cellular injury as well as an increase in both PKC and iNOS activities. The increase in PKC activity preceded that observed for iNOS. The ability of intraluminal PMA to decrease cellular integrity confirms and extends findings from in vivo experiments in which PKC activation via phorbol ester treatment was shown to mediate colonic mucosal and cellular injury (Fretland et al., 1990; Berin and Buell, 1995). Furthermore, we have recently demonstrated a direct cytotoxic action of PKC activators toward isolated colonic epithelial cells (Tepperman et al., 2000). PKC activation via PMA treatment appeared to mediate the increase in iNOS activity. This is suggested by the temporal relationship between the activities of the two enzyme systems. Furthermore, inhibition of PMA-mediated increases in PKC activity with GF109203X reduced the extent of cell injury. In contrast, the iNOS inhibitor 1400W reduced the extent of cell damage but did not affect PKC activity. This confirms previous studies, which have demonstrated that PMA administration could activate iNOS via a PKC-dependent mechanism (Hortelano et al., 1993; Fujihara et al., 1994; Zauli et al., 1996; Paul et al., 1997). Furthermore, the induction of NOS appears to play a role in the degree of cellular integrity because treatment with 1400W reduced the extent of cell injury in response to intraluminal PMA treatment.

Fig. 6. Effect of intracolonic instillation of PMA (3 mg kg⁻¹) on cell viability as estimated by Trypan blue dye uptake (A), PKC activity (B), and Ca²⁺-independent NOS activity (C) in cells harvested from the rat colon. Cells were isolated 1 to 6 h after PMA instillation. Cell were also taken from animals treated (0.5 ml) with the vehicle for PMA (20% ethanol). Values are displayed as means ± S.E.M. of six independent experiments. Asterisks (*) indicate significant differences between PMA and ethanol control animals as determined by the t test for paired data.

Fig. 7. Effect of PMA (3 mg kg⁻¹) on cell viability as assessed by Trypan blue dye uptake (A) and PKC activity in epithelial cells isolated from rat colon (B). Cells were harvested 4 h after PMA administration. In some experiments animals were pretreated 15 min before PMA administration with either the iNOS inhibitor 1400W or the selective PKC inhibitor GF 109203X (GFX). Values are displayed as means ± S.E.M. of seven experiments. Asterisks (+) indicate a significant increase over responses evident in cells harvested from animals treated with the vehicle for PMA (20% ethanol, control). Crosses (×) indicate significant decreases over the responses observed with PMA alone. Significance was determined by Duncan’s multiple range test.
Fig. 8. Effect of PMA treatment (3 mg kg⁻¹ intracolonically) on PKC isoforms in lysates of colonic epithelial cells. Cells were harvested 2 h after PMA treatment. A, displays representative Western immunoblots of isoforms in lysates of colonic epithelial cells. Cells were harvested 2 h from six independent experiments. Asterisks (*) indicate significant differences from the respective control as determined by the t test for paired data.

The present study revealed the presence of various PKC isoforms in cells harvested from the unstimulated as well as the LPS- and PMA-activated colon. The presence of multiple PKC isoforms has previously been demonstrated in the colonic epithelium of the rat (Jiang et al., 1995). PKC isoforms have been divided on the basis of the dependence of their activity on Ca²⁺ and their susceptibility to treatment with phorbol esters. The conventional PKCs, including PKC-α, are Ca²⁺-dependent and respond to phorbol esters; the novel PKCs (PKC-δ, PKC-ε) are Ca²⁺-independent but respond to phorbol esters; and the atypical PKCs such as PKC-ζ are independent of both Ca²⁺ and phorbol esters (Dekker and Parker, 1994). In the present study, LPS treatment resulted in activation of all isoforms examined, whereas PMA administration was associated with selective activation of the novel isoforms PKC-δ and PKC-ε. Previous studies in other tissues and cells have demonstrated that these isoforms are up-regulated in response to LPS treatment (Fujihara et al., 1994; Shapira et al., 1997). Studies directed at examining the isoforms activated in response to PMA have suggested that depending upon the tissue or cell type under investigation, the profile of PKC isoform activation can vary. However, many of these studies have suggested that PMA-mediated activation of PKC-ε may play an important role in the mediation of NOS induction and subsequent cellular injury (Fujihara et al., 1994; Keenan et al., 1997; Shapira et al., 1997). The functional role of these isoforms in the regulation of colonic cellular integrity is currently under investigation in this laboratory.

In conclusion, the present study has shown that LPS and PMA treatment in the rat can result in activation of colonic cellular PKC activity with subsequent induction of the Ca²⁺-independent NOS. The increase in iNOS is further associated with damage to the cells harvested from the colonic epithelium. Although a number of PKC isoforms may be involved in these responses, PMA treatment increased the translocation of PKC-δ and PKC-ε from cytosol to membrane, suggesting that these isoforms may play central roles in this process.

References


McKenna TM, Clegg JM and Williams TJ (1994) Protein kinase C is a mediator of lipopolysaccharide-induced vascular suppression in the rat aorta. Shock 2:84–89.