A Role for Protein Kinase C-δ in the Regulation of Ornithine Decarboxylase Expression by Oxidative Stress

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ABSTRACT

The expression of genes that regulate cell growth, such as ornithine decarboxylase (ODC), can be modulated by oxidant tumor promoters. Treatment of murine papilloma PE cells with H2O2 led to a transient induction of ODC enzyme activity, which could be blocked by calphostin, a nontoxic inhibitor of protein kinase C (PKC). Peak activity (11-fold) occurred 5–6 h after treatment, followed by a rapid decline. The increase in ODC activity was associated with an elevation of both ODC mRNA (3-fold) and protein (7-fold). Direct involvement of PKC in the regulation of ODC by oxidants was determined by stable transfection of PE cells with a dominant-negative PKC-δ mutant. PKC-δ activity was completely inhibited in response to H2O2 in cells overexpressing mutant PKC-δ compared with cells transfected with a blank plasmid. Induction of ODC mRNA, protein, and activity was also completely inhibited in cells expressing the PKC-δ mutant after H2O2 treatment. Activation of an ODC promoter-luciferase reporter construct by H2O2 was attenuated in mutant cells compared with control cells, further confirming that ODC is regulated transcriptionally by PKC-δ. However, fold-increases in ODC mRNA and protein were much less than the increase in activity, suggesting that ODC may also undergo posttranscriptional regulation in the presence of oxidants. Taken together, these studies provide new insight into the regulation of ODC by oxidants and suggest that PKC-δ may play a critical role in this regulation.

INTRODUCTION

Polyamines are small aliphatic molecules that play a key role in regulating cell proliferation (1). Polyamine biosynthesis is one of the first events to occur in proliferating cells, preceding both nucleic acid and protein synthesis (2). Although most of the specific functions of polyamines have not been identified, they are thought to fulfill structural and regulatory roles in nucleic acid and protein biosynthesis and function (3). ODC (EC 4.1.1.17) is the initial and rate-limiting enzyme in polyamine biosynthesis and is essential for cell proliferation. However, overexpression of ODC beyond some minimum threshold can induce cell transformation and tumor formation (4); indeed, abnormal levels of the enzyme have been detected in some animal and human cancers (2). ODC is also an important marker for tumor promotion in skin. Studies show that overexpression of ODC in mouse skin induces tumor promotion in the absence of typical tumor promoters, such as TPA (5). ODC activity is transiently induced by various mitogenic and toxic stimuli (6). During ODC induction, the increase in its mRNA is usually much less than the observed activity (7), suggesting that some regulation of ODC activity occurs posttranscriptionally. Isoelectric focusing analyses in various tissues and cells provide evidence for multiple forms of ODC (8–10), a further indication that the protein may undergo posttranslational modifications. Indeed, it has been demonstrated in several studies that ODC is phosphorylated in situ at serine and threonine residues (11). In RAW264 cells, phosphorylated ODC is more stable and exhibits a 50% higher catalytic efficiency than the unphosphorylated protein (12). Casein kinase II has been identified as one of the kinases that phosphorylates ODC in these cells; however, phosphorylation by this enzyme does not result in increased ODC activity (12).

Induction of ODC in murine keratinocytes treated with the tumor promoter TPA has been associated with PKC activation, and there is data that support transcriptional regulation of ODC by PKC-α (13). The current studies investigate the role of PKC-δ in the regulation of ODC by the oxidative agent H2O2. Oxidants derived from reactive oxygen species and organic hydroperoxides can contribute to tumor promotion, a process of selection and clonal expansion in which the expression of genes, such as ODC, that regulate cell growth are modulated in initiated cells (14). The pathways controlling the expression of such genes by oxidants in mammalian systems are not well defined. Oxidant tumor promoters also activate PKCs (15). PKC is a multienzyme family of serine/threonine kinases (16) that is classified into three groups: (a) classical PKCs (α, βI, βII, and γ), which are Ca2+-, phosphatidylserine, and diacylglycerol/TPA dependent; (b) novel PKCs (δ, ε, η, θ, and μ), which are phosphatidylserine and diacylglycerol/TPA dependent; and (c) atypical PKCs (ζ, τ, and A), which are Ca2+- and diacylglycerol/TPA independent. Regulation of ODC and tumor promotion is commonly studied in skin or cultured keratinocytes because skin is a good model for studying two-stage carcinogenesis. The present studies, performed in mouse papilloma cells, focused on the role of PKC-δ in the induction of ODC by H2O2, because although various PKCs are expressed in mouse epidermis, mainly the novel types, PKC-δ and PKC-η, are associated with keratinocyte differentiation (17, 18), a course that if deregulated could progress into a neoplastic phenotype in skin. Our choice to study PKC-δ was further influenced by the observations that expression of PKC-η protein in cultured keratinocytes is dependent on high Ca2+ concentrations, whereas PKC-δ expression is independent of Ca2+ levels (17). The mouse PE cells used in these studies were sustained in low Ca2+ levels to maintain them in a proliferative state. We find that H2O2 induces ODC activity severalfold and that this induction appears to be mediated by PKC-δ.

MATERIALS AND METHODS

Cell Culture and Treatment. Cell lines were maintained in a 37°C humidified environment containing 5% CO2 in air. Murine papilloma PE cells (19) were cultured in Eagle’s MEM without CaCl2 (BioWhittaker, Walkersville, MD) supplemented with Chelex (Bio-Rad, Hercules, CA)-treated fetal bovine serum (8%) and 0.05 mM CaCl2. Serum starvation was achieved by incubation in Eagle’s MEM supplemented with 0.5% Chelex-treated fetal bovine serum (8%) and 0.05 mM CaCl2 for at least 16 h prior to treatment with H2O2 or TPA in serum-free media.

Generation of Stably Transfected Cell Lines. Cells expressing dominant-negative PKC-δ (PEPKC-Δδ) were generated by transfection of the plasmid pSRD-DK376A (20) with lipofectamine (Life Technologies, Inc., Gaithersburg, MD) according to the supplier’s directions. Control cells were transfected with a blank plasmid (PEcontrol). G418-resistant clones were isolated by clonal dilution, expanded, and characterized.

ODC Assay. After treatment of confluent PE cultures, the cells were rinsed with pyridoxal phosphate (50 μg/ml)-containing PBS and harvested into...
Eppendorf tubes. The cells were subjected to three cycles of freezing on dry ice and thawing at 37°C. The cleared lysates were used to determine ODC activity as described previously (21).

**PKC Assay.** Treated cells were harvested and lysed in 20 mM Tris-Cl (pH 7.5), 1 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 4 mM diisopropylfluorophosphate, 1 mM sodium vanadate, 25 μg/ml leupeptin, 25 μg/ml pepstatin, 1% Triton X-100, and 50 mM mercaptoethanol for 30 min on ice. The cleared lysates were used to determine PKC activity by adding 2–4 μg of protein in an assay mixture containing 50 mM Tris-Cl (pH 7.5), 1 mM CaCl₂, 15 mM MgCl₂, 10 μM TPA, 0.25 mg/ml of phosphatidylserine, 50 μM ATP, 1 μCi of [γ-³²P]ATP, 2.5 mM DTT, and 50 μM of a PKC-δ pseudosubstrate region-derived peptide (22). The reactions were incubated at room temperature for 15 min and spotted onto phosphocellulose discs (Life Technologies, Inc.). The discs were washed twice with 1% phosphoric acid and twice with distilled water and analyzed by liquid scintillation. Nonspecific PKC activity was determined as described but in the absence of TPA or phosphatidylserine. The specific PKC activity was obtained by subtracting the nonspecific activity from the total activity.

**Immunoblot Analysis.** Cells were lysed in PBS by subjecting the extracts to three cycles of freezing in dry ice and thawing at 37°C. Cleared cell lysates (10 μg protein) were analyzed by Western blot analysis as described previously (23) with polyclonal antibodies specific to PKC-δ (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA). ODC protein expression was determined with a polyclonal ODC antibody.

**RNA Isolation and Northern Analysis.** Total RNA was isolated with RNA Stat-60 (Tel-Test, Inc., Friendswood, TX). RNA (20 μg/lane) was fractionated in formaldehyde-agarose gels and transferred onto Nytran membranes (Schleicher-Shuell, Keene, NH) according to the manufacturer’s directions. A mouse ODC cDNA probe was labeled with [γ-³²P]ATP and hybridized with washed and washed as shown above before the hybridization. Hybridization and washes were done as described previously (24). Hybridization was performed to a 24-bp oligonucleotide complementary to 18S RNA (5’-ACCGTATCTGATCGTCTCGAACC-3’) that was end-labeled with [γ-³²P]ATP by T4 kinase (Life Technologies, Inc.) was used to control for differences in loading and transfer efficiency.

**Transfections and Luciferase Assay.** PE cells were transiently transfected with 3 μg/ml of −4362/+131 ODC-luciferase (25), 0.2 μg/ml of pRLTK (Promega Corp., Madison, WI), and 12 μl of lipofectamine (Life Technologies, Inc.) according to the manufacturer’s directions. Cells were treated with 200 μM H₂O₂ and luciferase activity was determined 6 h after treatment with the dual-luciferase reporter assay system (Promega). Luciferase activities were determined with a Berthold LB9505 luminometer.

**Adenoviral Vectors and Infection.** Constitutively active PKC-δ (20) was subcloned into the modified adenoviral shuttle vector pAdEG1 (26), and virus was purified essentially as described (26–28). PE cells were infected with viruses (100 plaque-forming units/cell) for 2–4 h in serum-free media. Infection media was then replaced with normal growth medium, and cells were allowed to grow for 24 h, after which they were treated with H₂O₂ and analyzed for ODC activity. The fluorescence of green fluorescent protein was analyzed for ODC activity. The fluorescence of green fluorescent protein was detected by one-way ANOVA, followed by Bonferroni’s multiple corrections. A mouse ODC cDNA probe was labeled with [γ-³²P]ATP by T4 kinase (Life Technologies, Inc.) was used to control for differences in loading and transfer efficiency.

**Statistical Analyses.** Statistical significance of the differences in the means was assessed by way ANOVA, followed by Bonferroni’s multiple comparison test.

**RESULTS**

**H₂O₂ Induces ODC Activity in PE Cells.** ODC activity is a hallmark for the action of tumor promotion, in vivo and in vitro. PE cells, a murine keratinocyte cell line derived from mouse skin, are established in culture and do not normally express high levels of ODC activity. However, treatment with H₂O₂ results in a transient increase in ODC activity, as measured by an increase in luciferase activity (21). This increase is not specific to PKC or PKC-δ alone, as it is also observed in cells transfected with PKC-δ pseudosubstrate region-derived peptide (22). The rise in ODC activity is correlated with an increase in the expression of ODC protein, as measured by Western blot analysis (23). The transient increase in ODC activity is not due to the presence of PKC or PKC-δ itself, as it is observed in both control and transfected cells (24).

**Fig. 1.** Induction of ODC by H₂O₂ and inhibition with calphostin. A, PE cells were treated with graded concentrations of H₂O₂ for 5 h, and ODC activity was determined as described in “Materials and Methods.” B, PE cells were pretreated with 1 μM calphostin for 45 min before incubation with 200 μM H₂O₂ for 5 h, and measurement of ODC activity was determined. Data are the means of triplicate plates assayed in triplicate; bars, SD.

**Role of PKC on Activation of ODC by H₂O₂.** The following set of studies attempted to identify the molecular pathway by which oxidants activate ODC. The fact that H₂O₂ activates PKC (30), coupled with our observation that H₂O₂ induces ODC activity, led us to question whether PKC plays a role in this process. Pretreatment of cells with 1 μM calphostin, a nonspecific PKC inhibitor, prior to treatment with 200 μM H₂O₂ resulted in complete inhibition of ODC activity compared with cells treated with H₂O₂ only (Fig. 1B). Calphostin alone had no effect on constitutive levels of ODC activity.

To further probe the role of PKC types in the regulation of ODC by oxidants, cells were stably transfected with a dominant-negative PKC-δ construct and characterized by immunoblot analysis and PKC activity measurements. PKC-δ was found to be increased in double phosphorylated and unphosphorylated proteins as determined by immunoblot analysis of control and transfected cells (Fig. 2A). As demonstrated in Fig. 2A, Lanes 2 and 3, there was a 3-fold increase in mutant protein in PEPKC-δDN cells compared with native protein in PEcontrol cells. The dominant-negative PKC-δ is mutated in its ATP-binding site and therefore lacks the ability to autophosphorylate and activate itself. However, a slower migrating phosphorylated mutant was still obtained in PEPKC-δDN cells. We speculate that this outcome is likely attributable to the fact that wild-type PKC-δ and its kinase-inactive mutant are both phosphorylated on tyrosine residues by tyrosine kinases (30). PKC activity, as determined with a peptide designed against the PKC-δ pseudosubstrate region, was increased 3-fold in cell extracts isolated from H₂O₂-treated PEcontrol cells. This activity was completely inhibited in extracts from H₂O₂-treated PE PKC-δDN cells (Fig. 2B), consistent with a functional loss of PKC-δ. PKC activity in extracts isolated from TPA-treated PEcontrol cells was increased 2-fold, but this activity was only decreased by 50% in PEPKC-δDN cells after treatment with TPA. Presumably, PKC activity was partially inhibited in extracts from TPA-treated PE PKC-δDN cells because, although the PKC-δ pseudosubstrate peptide has high specificity for PKC-δ, it is not absolute and can be phosphorylated by other PKC types. Also, in the presence of TPA, the concentration of the dominant-negative PKC-δ in PEPKC-δDN cells may not be high enough to overcome the particularly robust activation of multiple types of PKCs.

**Mediation of PKC in the Induction of ODC Activity by H₂O₂.** The time course for the induction of ODC activity by H₂O₂ was investigated in PEcontrol and PE PKC-δDN cells after treatment with 200 μM H₂O₂. A transient induction in ODC activity was observed, as shown in Fig. 1A, consistent with a 25-fold increase in the tumor promoter TPA (100 ng/ml), a well-established inducer of ODC activity (data not shown).
observed in PE<sub>control</sub> cells, with maximal ODC activity observed between 5 and 7 h of treatment (Fig. 3A), followed by a rapid decline in enzymatic activity. This transient induction of ODC by various stimuli is attributed to a rapid degradation of ODC through an antizyme-dependent mechanism (31). ODC activity was not increased at any time point in PE<sub>PKC-δ</sub>-DN cells upon treatment with H<sub>2</sub>O<sub>2</sub>, suggesting that PKC-δ may be required for induction of ODC expression by H<sub>2</sub>O<sub>2</sub> (Fig. 3A). By contrast, a partial inhibition of ODC induction in response to TPA was seen in PE<sub>PKC-δ</sub>-DN cells compared with PE<sub>control</sub> cells at a low dose (20 ng/ml; Fig. 3B). No inhibition was seen at a higher dose (100 ng/ml) of the phorbol ester (data not shown). This result suggests that for TPA, unlike H<sub>2</sub>O<sub>2</sub>, inhibition of PKC-δ in PE<sub>PKC-δ</sub>-DN cells has limited impact upon ODC induction and that elevation of other PKC enzymes is important in signaling for enzyme induction.

To further confirm that PKC-δ is a mediator in the induction of ODC by H<sub>2</sub>O<sub>2</sub>, PE<sub>PKC-δ</sub>-DN cells were infected with virus expressing a constitutively active PKC-δ attached to green fluorescent protein. This approach was taken to achieve a high level of constitutively active PKC-δ in PE<sub>PKC-δ</sub>-DN cells. About 80–90% of the cells expressed constitutively active PKC-δ, as determined by the presence of green fluorescent protein (Fig. 4B). The distribution of PKC-δ was concentrated in the perinuclear region, although some protein was also observed in the cytoplasm. In cells infected with virus containing green fluorescent protein only (positive control), the protein was expressed diffusely throughout the cells (Fig. 4C). Overexpression of constitutively active PKC-δ in PE<sub>PKC-δ</sub>-DN cells increased basal ODC activity 4-fold in untreated cells. Moreover, this maneuver restored ODC inducibility as activity was further enhanced 8-fold after H<sub>2</sub>O<sub>2</sub> treatment (Fig. 4D).

ODC Gene and Protein Expression in PE<sub>control</sub> and PE<sub>PKC-δ</sub>-DN Cells after H<sub>2</sub>O<sub>2</sub> Exposure. Having established that PKC-δ is important in the regulation of ODC enzyme activity by H<sub>2</sub>O<sub>2</sub>, the next step was to determine whether the effect on induction was transcriptional and/or posttranscriptional. The kinetics of appearance of ODC message and protein levels in cells treated with H<sub>2</sub>O<sub>2</sub> was analyzed by Northern and Western analyses. Significant increases in ODC mRNA levels in PE<sub>control</sub> cells were initially observed after 4 h of treatment, and peak message levels (3-fold increase) were maintained up to 7 h of treatment (Fig. 5). No induction of ODC mRNA occurred in H<sub>2</sub>O<sub>2</sub>-treated PE<sub>PKC-δ</sub>-DN cells (Fig. 5).

Treatment of PE<sub>control</sub> cells with TPA (positive control) resulted in a time-dependent increase in ODC protein with maximal levels observed at 6 h (14-fold), as determined by immunoblot analysis (Fig. 6A). A similar time-dependent, albeit smaller increase in maximal ODC protein (7-fold at 6 h) was observed in PE<sub>control</sub> cells after H<sub>2</sub>O<sub>2</sub> treatment (Fig. 6B). However, no increase in ODC protein was observed in PE<sub>PKC-δ</sub>-DN cells (Fig. 6C), except for trace elevation of ODC protein after 7 h of H<sub>2</sub>O<sub>2</sub> treatment, which did not correspond to any increase in ODC activity at the same time point.

DISCUSSION

ODC regulation has been the subject of numerous investigations in different models of multistage carcinogenesis but most commonly in skin. For instance, transgenic animals that express both elevated ODC and activated ras develop spontaneous skin tumors in the absence of carcinogens or tumor promoters, suggesting a cooperation between these genes during tumor development (32). The mechanism for such a cooperation, however, is not clear. Nonetheless, this and other observations point to the importance of deregulation of ODC in the neoplastic process.

A direct role for ODC in tumor promotion is evident in studies where targeted expression of high levels of ODC to mouse skin produced tumors after initiation with a carcinogen, in the absence of administration of a tumor promoter (5). The present studies examined

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**Fig. 2.** Characterization of cells expressing dominant-negative PKC-δ. A, equal amounts of denatured protein (20 μg) from parental PE cells and cells transfected with a blank plasmid or dominan-negative PKC-δ (PKC-δ<sub>DN</sub>) were resolved by SDS-PAGE (8%), and transferred proteins were immunoblotted with anti-PKC-δ serum as described in “Materials and Methods.” B, cells were treated with 200 μg H<sub>2</sub>O<sub>2</sub> for 30 min or 40 ng/ml TPA for 15 min, and PKC activity was determined with the PKC-δ-specific peptide substrate as described in “Materials and Methods.” Data are shown as means of triplicate cultures; bars, SD; *, differ from control, P < 0.05. n.d., not detected.

![Image](cancerres.aacrjournals.org)
the regulation of ODC by H$_2$O$_2$, primarily because oxidants are known tumor promoters in vivo, and secondly, the precise mechanisms by which oxidants cause promotion are not clear. The current studies demonstrate that ODC activity is induced in murine keratinocytes by H$_2$O$_2$, an action common to most, if not all, tumor promoters in skin. There appear to be several mechanisms by which oxidants can trigger signal transduction pathways leading to enhanced gene expression. For instance, butylated hydroperoxide and H$_2$O$_2$ activate the mitogen-activated protein kinase cascade (33, 34), a critical signaling pathway for cellular proliferation. This well-characterized pathway is stimulated by growth factors, mitogens, and stress responses and is initiated by tyrosine kinases that sequentially activate ras, c-raf, and other downstream kinases. Deregulation of this pathway, as might occur in ras-transformed cells, is associated with ODC overexpression (35).

Another important signaling pathway in the cell, the multi-enzyme PKC family, which mediates many of the actions of the potent tumor promoter TPA, can also be activated by H$_2$O$_2$. However, H$_2$O$_2$ activates PKC-δ by a mechanism that differs from TPA activation of this kinase. Unlike TPA, stimulation of cells with H$_2$O$_2$ does not require PKC-δ translocation to the membrane for activation, and the activity of PKC-δ isolated from H$_2$O$_2$-treated cells can be measured independently of lipid cofactors (36). Investigations into the mecha-

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**Fig. 4.** Infection of PE$_{PKC-δDN}$ with virus containing constitutively active PKC-δ attached to green fluorescent protein. PE$_{PKC-δDN}$ cells were infected with no virus (A, $\times400$) or virus expressing constitutively active PKC-δ attached to green fluorescent protein (B, $\times200$), or virus expressing green fluorescent protein (GFP) only (C, $\times200$). D. PE$_{PKC-δDN}$ cells infected with no virus (■) or virus expressing constitutively active PKC-δ (▴) were treated with 200 μM H$_2$O$_2$ for 5 h, and ODC activity was determined. Data are the means of triplicate plates assayed in triplicate; bars, SD.

**Fig. 5.** Northern blot analysis of ODC mRNA in PE control and PE$_{PKC-δDN}$ cells. Total RNA was isolated from PE control (A) and PE$_{PKC-δDN}$ (B) cells at the indicated times after H$_2$O$_2$ treatment. RNA (20 μg) was fractionated on a 1.2% agarose/formaldehyde gel, transferred to Nytran membranes, and hybridized with a random-labeled ODC cDNA probe. The 18S signal was used as a control for variations in loading and transfer. Levels of RNA were quantified with a Fuji BAS1000 phosphorimaging system, and normalized RNA signals were plotted.

**Fig. 6.** Western blot analysis of ODC protein in PE control and PE$_{PKC-δDN}$ cells. Total cell lysates were isolated from PE control cells treated with TPA and H$_2$O$_2$ (A and B) or PE$_{PKC-δDN}$ treated with H$_2$O$_2$ (C) at the indicated times. Denatured proteins (30 μg) were resolved by SDS-PAGE (8%), transferred onto nitrocellulose membranes, and immunoblotted with anti-ODC antibody. The blots were scanned, and the bands were quantitated with the MacBas v.2.2 software (Fuji Photofilm Co., Ltd., and Kohshin Graphic System, Tokyo, Japan).
nisms of activation of PKC indicate that oxidants may modify cysteine residues on several PKCs, leading to their activation (37). Other studies show tyrosine phosphorylation of major PKC isoforms, including PKC-δ, in the presence of H2O2, and suggest that this may be a mechanism of activation by this oxidant (30).

Cellular expression of the dominant-negative PKC-δ completely prevented the inducibility of ODC by H2O2, suggesting that this PKC isoform specifically regulates ODC in response to oxidants and perhaps other actions of oxidants in mouse skin. However, there are contrary views as to the role of PKC-δ in tumor promotion and cell transformation. For instance, whereas a dominant-negative PKC-δ mutant inhibited 6-str-induced transformation and platelet-derived growth factor-BB-mediated anchorage-independent colony formation in NIH3T3 cells (38), expression of this mutant in Y1 fibroblasts induced c-src-mediated transformation of these cells and TPAb-induced anchorage-independent colony formation (39). These data reflect how little is known on the physiological role of PKC-δ. The complexity of PKC-δ regulation and function is further demonstrated by recent studies that find PKC-δ to be a substrate for c-src, which phosphorylates this kinase at tyrosine residues and promotes its degradation (40).

Overexpression of PKC-δ in vivo and in vitro has also been shown to inhibit tumor promotion and induce apoptosis, respectively (41, 42), supporting a role for PKC-δ as a tumor suppressor. However, the physiological relevance of overexpressing this protein remains to be determined because overexpression may overwhelm endogenous protein levels, leading to nonspecific PKC-δ localization and substrate specificity. This consideration is especially important in light of very recent data showing differential localization of PKC-δ to cellular, nuclear, or Golgi membranes in response to different agonists (43). Such a differential localization may influence substrate specificity of PKC-δ, and hence, its biological response, especially if there is colocalization with a substrate. Unfortunately, knowledge of the physiological substrates of PKC-δ that may aid in understanding its diverse physiological response is scant.

The fact that we demonstrate an increase in ODC mRNA (3-fold) in response to H2O2 that does not correspond to an increase in ODC protein (7-fold) or enzyme activity (12-fold) suggests the additional involvement of posttranscriptional regulation of ODC by oxidants. It is well documented that ODC undergoes rapid degradation upon stimulation; it has also been shown that ODC can be phosphorylated in situ and in vitro and that phosphorylated ODC is more stable and has a higher catalytic activity than the unphosphorylated protein (12). Although we report that PKC-δ is required for the induction of ODC activity by oxidants and although our data support a role for transcriptional regulation, we cannot rule out the possibility of posttranscriptional effects, such as phosphorylation of ODC by PKC-δ, to explain the full increase in ODC activity.

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