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Phorbol Ester Synergistically Increases Interferon- γ -Induced Nitric Oxide Synthesis in Murine Microglial Cells

Key Words

Microglia
Astrocyte
Nitric oxide
Immunologic nitric oxide synthase
Protein kinase C

Abstract

The effect of phorbol ester on the synthesis of nitric oxide (NO) in murine microglial cells was examined. Phorbol 12-myristate 13-acetate (PMA), a protein kinase C (PKC) activator, alone had no effect, whereas PMA with recombinant interferon (rIFN)- γ synergistically increased NO synthesis in murine microglial cells. The maximal effect of PMA on NO synthesis increase always fitted with the range for full activation of PKC in these cells. The increase of NO synthesis was reflected as increased amount of immunologic NO synthase (iNOS) mRNA detected by Northern blotting. Treatment with PKC inhibitors such as staurosporine or polymyxin B decreased rIFN- γ -plus-PMA-stimulated NO synthesis. Further, prolonged incubation of the cells with PMA, which down-regulates PKC activity, abolished the synergistic cooperative effect with IFN- γ . N^G-monomethyl-L-arginine monohydrate, an analogue of L-arginine, and arginase inhibited rIFN- γ -plus-PMA-induced NO production in murine microglial cells. On the basis of these observations, we conclude that PKC might not be involved in the expression of iNOS, but instead, might be involved in the posttranscriptional modification of iNOS mRNA.

Introduction

Nitric oxide (NO) is an important intracellular and intercellular regulatory molecule with functions as diverse as vasodilation, neural communication and host defence [1-3]. The biological ability of NO in vivo depends largely on its chemical reactivity towards certain target molecules and on the composition of the physiological microenvironment into which it is released. The production of NO has been identified as a key event in the expression of the antimicrobial and antitumor functions of activated macrophages.

NO is produced from oxidation of a guanidino nitrogen of L-arginine by NO synthase (NOS) [4]. Activation of NOS yields the short-lived nitrogen-centered NO radical (referred to as NO[•]) [5-7]. NO reacts with itself, oxygen and water to generate the nitrogen dioxide radical and the accumulating end products, nitrite and nitrate [8].

There are three isoforms of NOS in mammalian tissues [1, 2]. Neural and endothelial cells contain two different, although related, constitutive enzymes that require Ca²⁺ and calmodulin to be active, whereas the

isoenzyme expressed in macrophages after stimulation with cytokines and endotoxins is said to not require these cofactors

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[2, 3], although these agents may be tightly bound to immunologic NOS (iNOS) or a subunit.

Recent reports have shown that murine brain microglial cells can also synthesize NO in response to interferon (IFN)- γ and bacterial cell wall components such as lipopolysaccharide (LPS) [9]. It has also been reported that NO mediates the toxoplasmatic activity in murine microglial cells in vitro [10]. Microglial cells have a macrophage-like function in the central nervous system (CNS) and express a variety of immunological characteristics such as homeostatic control and defence of the organism against infection and injury [4].

However, the exact mechanism of the induction of NO synthesis in the CNS is not fully understood. In this study, therefore, we decided to investigate whether protein kinase C (PKC) is associated with NO synthesis in murine microglial cells.

We show that phorbol 12-myristate 13-acetate (PMA), a biologically active phorbol ester, synergistically induces NO synthesis in murine microglial cells which have been treated with recombinant IFN- γ (rIFN- γ), but PMA has no effect on NO synthesis by itself. PKC inhibitors such as staurosporine (STSN) or polymyxin B abolish the synergistic effect of PMA with IFN- γ on NO synthesis. In addition, prolonged incubation of microglial cells with PMA, which down-regulates PKC activity, decreases NO synthesis induced by IFN- γ plus PMA. These results demonstrate that PKC might not be directly involved in iNOS expression but, instead, might be involved in the post-transcriptional modification of iNOS mRNA or post-translational regulation of iNOS in microglial cells.

Materials and Methods

Mice

The original stock of BALB/c mice was purchased from The Jackson Laboratory (Bar Harbor, Me., USA), and the mice were maintained in the Department of microbiology and Immunology, Wonkwang University School of Medicine (Iri, Korea). To obtain microglial cells, mice were used at 2-5 days of age.

Enzymes and Chemicals ■ Murine rIFN- γ (1×10^7 U/mg) was purchased from Genzyme (Munich, Germany). Dulbecco's modified Eagle's medium (DMEM), LPS (phenol-extracted *Salmonella enteritidis*), PMA, STSN, polymyxin B, N-(1-naphthyl)ethylenediamine dihydrochloride, sodium nitrite and sulfanilamide were purchased from Sigma (St. Louis, Mo., USA). Tissue culture flask and 96-well tissue culture plates were purchased from Nunc (Naperville, Ill., USA). Hank's balanced salt solution (HBSS), fetal calf serum (FCS) and other tissue culture reagents were purchased from Life Technologies (Gaithersburg, Md., USA).

Preparation of Glial Cell Cultures

Primary glial cell cultures were established from brains of newborn, pathogen-free BALB/c mice (School of Medicine, Wonkwang University) as described elsewhere [11]. After removal of the meninges, brains of newborn BALB/c mice were disaggregated using nylon sieves (120 μ m pore size) and seeded in 50-ml tissue culture flasks in DMEM containing 20% heat-inactivated FCS. The inoculation volume was 4 ml/flask and contained 60 mg of dissociated brain materials. Growth medium was replaced on the 3rd and 6th day with DMEM containing 10% FCS. On day 10, subcultures were made by treating the cells with 0.25% trypsin/0.05% EDTA and, after washing, 1×10^6 cells/ml were plated in either a 50-ml tissue culture flask or 24-well tissue culture plate in DMEM with 5% FCS.

Isolation of Microglial Cells

To isolate microglia from cultured glial cells, culture flasks were vigorously agitated for 1 h on a rotary shaker (Shaker SI 100; Pharmacia Diagnostics) at 800 rpm, filtered through a 20- μ m nylon mesh, plated in a fresh flask, and attached for 15 min at 37°C followed by extensive washing with HBSS [12]. Adherent cells were harvested with a rubber policeman and resuspended in DMEM containing 10% heat-inactivated FCS. Dispersed cells were then plated in 24-well tissue culture plates at a concentration of 2×10^5 cells/well. The adherent cells were identified as microglial cells and astrocytes using immunofluorescent staining with monoclonal anti-Mac-1 antibody [13] and anti-glial fibrillary acidic protein (GFAP) antibody [12].

Astrocyte Cultures

Astrocytes were prepared from brains of newborn BALB/c mice as described elsewhere [11]. After 3 days in subculture, more than 95% of these first-passage cells were positive for GFAP as indicated in immunofluorescent studies that made use of anti-GFAP antiserum. Because GFAP is a marker for astrocytes in brain tissue, more than 95% of the first-passage cells are astrocytic cells.

Measurement of Nitrite

Microglial cells (2×10^5 cells/well) or astrocytes (2×10^5 cells/well) were incubated with reagents as detailed in the Results section. Supernatants were collected after 48 h incubation, and the nitrite concentration in the medium was measured by a microplate assay method as previously described [14]. Briefly, 100- μ l aliquots were removed from conditioned medium and incubated with an equal volume of the Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)ethylenediamine dihydrochloride/2.5% H₃PO₄) at room temperature for 10 min. The absorbance at 540 nm was determined in a Titertek Multiskan (Flow Laboratories, North Ryde, Australia). NO₂⁻ was determined using sodium nitrite as standard. Cell-free medium alone contained 5–8 μ M NO₂⁻; this value was determined in each experiment and subtracted from the value obtained with cells.

Preparation of Probe

To detect NOS mRNA transcripts, sense and antisense oligonucleotide primers specific for the coding regions of that gene were synthesized using conventional technology. The following oligonucleotide primers were used: forward primer, 5'-GGCCTTGGCTCCAG-CATGTAC-3', 1856 through 1976; reverse primer, 5'-GCTGCC-GCTCTCATCCAGAAC-3', 2395 through 2415. The numbers represent the nucleotide numbers on the complementary strands of each cDNA sequence [15,16]. Total cellular RNA (5 μ g) from rIFN- γ - and PMA-stimulated murine microglial cells was used as a template.

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plate and the single-stranded cDNA was synthesized with downstream antisense primers by reverse transcriptase. cDNA was amplified in a 12- μ l reaction mixture by a Dae Han Medical Co. DNA thermal cycler (Seoul, Korea) using Taq polymerase in 45 cycles of 5 s denaturation at 94°C, 5 s annealing at 55°C and 20 s synthesis at 72°C. PCR products were examined on 1% Treatment

agarose gels. For the DNA sequence analysis PCR products were gel-purified, treated with T4 polynucleotide kinase, and then with the Klenow fragment of DNA polymerase I. The products were subcloned into the *EcdKV* site of the pBluescript II KS(-) plasmid. One microgram of plasmid DNA was radiolabeled by random priming with [α - 32 P]dCTP. The resultant specific activity was approximately 1×10^8 cpm/ μ g and was used at 1×10^7 cpm/blot.

UNA Extraction and Northern Blotting

Total RNA was prepared by the LiCl-urea method [17], electrophoresed in 1.2% agarose-formaldehyde gels and transferred to nylon membranes by capillary action in $20 \times$ SSC ($1 \times$ SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.2). After prehybridization, the filters were hybridized with random [α - 32 P]dCTP-labeled probes with specific activities of $1-5 \times 10^8$ cpm/ μ g in 10% dextran sulfate, 50% formamide, $4 \times$ SSC, $1 \times$ Denhardt's solution and 10 μ g/ml salmon sperm DNA for 24 h at 42°C. The filters were then washed, dried, and examined by autoradiography.

Table 1. Effects of rIFN- γ and/or PMA on nitrite production by murine microglia or astrocytes
NO $_2$ secretion, μ M microglia astrocyte

Medium	&a &am
rIFN- γ	1 t p ; 1
rIFN- γ plus PMA	15 t ; 5
	-

Microglia and astrocyte fractions (2×10^5 cells/well) were isolated as described in Materials and Methods. The fractions were incubated for 48 h in medium alone or in medium containing rIFN- γ (5 U/ml) or rIFN- γ (5 U/ml) plus PMA (200 nM). Nitrite was measured by the method of Griess. Values are means $\pm$ SD of three experiments.

Results

Effect of rIFN- γ and/or PMA on NO Synthesis by Microglia or Astrocyte

We found that after 48 h incubation, murine microglial cells synthesized far more NO $_2$ than murine astrocytes upon stimulation with IFN- γ plus PMA (table 1). Though astrocytes are glial cells, they might have different morphological or immune influences in induction of NO synthesis not shared by microglia. Compared with the cells cultured in medium only, rIFN- γ -treated cells produced large amount of nitrite in response to PMA (table 2). PMA or LPS synergistically increased NO synthesis from murine microglial cells which had been treated with rIFN- γ but had no effect on the cells not pretreated with IFN- γ (table 2). When PMA was added to rIFN- γ -treated cells, the synergistic effect was always maximal at 200 nM PMA (fig. 1). The release of NO elicited by PMA fitted with the range of concentration required to activate PKC by phorbol esters. As table 2 shows, the optimal time to increase NO production by PMA was 6 h after rIFN- γ treatment. To determine whether the increased nitrite secretion is correlated with the iNOS mRNA level, we examined iNOS mRNA content. Total RNA was isolated from cultured cells, separated by denaturing agarose gel electrophoresis, and analyzed by Northern hybridization (fig. 2). Expressed iNOS mRNA levels were correlated with the amounts of nitrite secreted into the medium.

Table 2. Time-dependent effects of PMA on rIFN- γ -induced NO synthesis in murine microglial cells

Treatment		NO $_2$ secretion, μ M	
t			
PMA	LPS	rIFN- γ (-)	rIFN- γ (+)
-	-	<5	15 $\pm$ 1.2
-	+	7 $\pm$ 1.4	57 $\pm$ 8.2
+ (-6h)	-	ND	12 $\pm$ 1.0
+ (0h)	-	ND	24 $\pm$ 0.8
+ (+3h)	-	ND	30 $\pm$ 2.0
+ (+6 h)	-	<5	48 $\pm$ 4.1
+ (+9 h)	-	ND	36 $\pm$ 5.6
+ (+12h)	-	ND	28 $\pm$ 5.1

Murine microglial cells (2×10^5 cells/ well) were cultured in either medium alone or in medium containing PMA (200 nM) in the presence or absence of rIFN- γ (5 U/ml) at various times after incubation. NO release was measured by the method of Griess (nitrite), 48 h after incubation. Values are means $\pm$ SD of three experiments. ND = Not determined.

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Fig. 1. PMA in a dose-dependent fashion induces NO synthesis from rIFN- γ -treated murine microglial cells. Cells (2×10^5 cells/ well) were incubated for 6 h in medium containing rIFN- γ (5 U/ml), stimulated with PMA and incubated in a CO₂ incubator for 42 h. NO synthesis was measured by the method of Griess (nitrite). Values are means $\pm$ SD of three experiments.

0.2 2 20 200 2000

PMA concentration (nM)

Fig. 2. A Effects of PMA on the synthesis of NO in rIFN- γ -treated murine microglial cells. Cells (2×10^5 cells/well) were incubated for 6 h in medium containing rIFN- γ (5 U/ml), stimulated with PMA and incubated in a CO₂ incubator for 42 h. NO synthesis was measured by the method of Griess (nitrite). B Effects of PMA on the expression of the iNOS gene in rIFN- γ -treated murine microglial cells. Cells (2×10^5 cells/well) were incubated for 6 h in medium that contained rIFN- γ (5 U/ml), stimulated with PMA and further incubated in a CO₂ incubator for 6 h and then analyzed for the expression of the iNOS gene by Northern blotting.

None IFN PMA IFN IFN
+PMA +PMA <6h) (Oh)

f+fff

I 28S •-iNOS
18S

p-actin

rIFN- γ caused only modest, whereas rIFN- γ plus PMA caused marked increases in iNOS mRNA. PMA alone had no effect on the expression of the iNOS gene.

Suppression of NO Synthesis by STSN or Polymyxin B in rIFN-γ plus-PMA-Treated Microglial Cells To further analyze the influence of PKC on induction of NO synthesis, we examined the influence of PKC inhibitors such as STSN or polymyxin B in rIFN-γ plus-PMA-treated microglial cells. Adding STSN or polymyxin B to the rIFN-γ plus-PMA-treated cells decreased the synergistic effects of PMA on NO synthesis (table 3), but did not decrease the NO synthesis induced by rIFN-γ alone. Further, treatment of the cells with PMA for 6 h before the stimulation with IFN-γ revealed no synergistic

cooperation between IFN-γ and PMA in the synthesis of NO. This phenomenon might be caused by down-regulation of PKC, because it is well-known that prolonged treatment of cells with phorbol ester leads to a substantial loss of PKC enzyme activity for several hours. These effects of PKC inhibitors and prolonged incubation of the cells with PMA further suggest that the signal of NOS induction by IFN-γ may be regulated independently of PKC activation in murine microglial cells.

Inhibition of NO Production by NG-Monomethyl-L-Arginine (NMMA) and Arginase in IFN-γ plus-PMA-Treated Murine Microglial Cells To determine if the signaling mechanism in PMA-induced NO production is also involved in the Z⁺ argi

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Table 3. Effects of STSN or polymyxin B on NO synthesis induced by rIFN-γ or rIFN-γ plus PMA

Treatment	PKC inhibitors		NO secretion (μM)
	STSN	polymyxin B	
Medium only	—	—	<5
rIFN-γ	—	—	15.0±1.2
	+	—	11.2±3.4
	+	+	13.4±4.3
rIFN-γ plus PMA	+	—	48.5±5.1
	+	+	16.4±3.7
	+	+	18.1±3.3

Murine microglial cells (2 x 10⁵ cells/well) were cultured for 6 h either in medium alone or in medium that contained rIFN-γ (5 U/ ml) with STSN (200 nM) or polymyxin B (100 U/ml). The cells were then treated with PMA (200 nM) and incubated in a CO₂ incubator for 48 h. NO release was measured by the method of Griess (nitrite). Values are means ± SD of three experiments.

fig. 3. Effects of NMMA or Arginase on NO synthesis from murine microglial cells. Microglial cells were treated with 5 U/ml rIFN- γ plus 200 nM PMA. Then, the cells were incubated with different concentrations of NMMA or arginase at 37°C for 48 h. NO release was measured by the method of Griess. Data are means $\pm$ SD of triplicates.

nine-dependent pathway in microglial cells, microglial cells were incubated for 6 h in the presence of IFN- γ plus NMMA or IFN- γ plus arginase. The cells were then treated with PMA and cultured for 42 h. The production of nitrite by IFN- γ plus PMA in microglial cells was progressively inhibited with increasing amounts of NMMA and arginase (fig. 3).

Discussion

Even though the brain has been considered to be an immunologically privileged organ, recent reports indicate that certain brain cells are involved in immunological processes. It has been reported that NO in the CNS can act as a defence mechanism against microbes, parasites or tumor cells [9-13]. However, the signal transduction mechanism of NO production in the CNS, especially in microglia, is unknown.

Microglia and astrocytes, two major glial cell types, were purified, cultured, stimulated with rIFN- γ , PMA, LPS, rIFN- γ plus PMA, or rIFN- γ plus LPS, and NO₂ was then measured. The result shows that only microglia can induce NO production. It is reported that NO secretion may be regulated through a PKC pathway [18]. However, at which point PKC is involved in NO synthesis is not My understood. In this study, PMA, a PKC activator,

did not induce NO synthesis but, instead, PMA in combination with IFN- γ synergistically increased NO synthesis in microglial cells. This study demonstrates that PKC alone is not sufficient for the expression of NOS but is involved in synergistic cooperation with IFN- γ as a cosignal. PMA had a maximal effect on NO synthesis at a concentration of 200 nM in IFN- γ -treated murine microglial cells. PMA exerted its maximal effect 6 h after treatment with IFN- γ . Prolonged incubation of microglial cells with PMA abolished the synergistic cooperative effect of PMA with IFN- γ . This may be the result of PKC down-regulation. If PKC is involved directly in the induction of iNOS in murine microglial cells by rIFN- γ , down-regulation of PKC could affect IFN- γ -induced NO synthesis. However, preincubation of the cells with PMA did not affect NO synthesis by IFN- γ , and only altered the synergistic cooperation between IFN- γ and PMA. When the cells were treated with PKC inhibitors such as STSN or polymyxin B, NO synthesis was greatly diminished.

The results of this study suggest that PKC may provide a second signal for synergistic induction of NO synthesis in murine microglial cells already induced to express iNOS mRNA by IFN- γ . It is crucial to define the point at which PKC might be involved in NO synthesis. It is possible that the induction of NO synthesis in macrophages requires protein synthesis [5], but the activity of the enzyme may be affected by posttranslational modifica-

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tion. This may be the case for the constitutive enzyme expressed in brain. A recent study has shown that phosphorylation of this enzyme by PKC increases its activity, whereas phosphorylation by calcium-calmodulin-dependent kinase results in reduced activity [19]. In contrast, another report suggested that brain NOS cannot be phosphorylated by PKC, but can be by protein kinase A, which, however, does not alter NOS activity [20].

It is also possible that, following IFN- γ stimulation, PKC is involved in the induction of transcription of the iNOS gene. The gene has recently been cloned from activated RAW 264.7 cells [15] and the regulation of its transcription is now being investigated.

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References

- Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991;43:109-142.
- Lowenstein CJ, Snyder SH: Nitric oxide, a novel biologic messenger. *Cell* 1992;70:705-707.
- Yui Y, Hattori R, Kosuga K, Eizawa H, Hiki K, Kawai C: Purification of nitric oxide synthase from macrophages. *J Biol Chem* 1991;266:12544-12547.
- Pow DV, Perry VH, Morris JF: Microglia in the neurohypophysis associate with and endo-cytose terminal portions of neurosecretory neurons. *Neuroscience* 1989;33:567-578.
- Marietta MA, Yoon PS, Iyengar R, Leaf CD, Wishnok JSC: Macrophage oxidation of L-arginine to nitrite and nitrate: Nitric oxide is an intermediate. *Biochemistry* 1988;27:8706-8711.
- Hibbs JB Jr, Taintor R, Varrin Z, Rachlin C: Nitric oxide: A cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun* 1988;157:87-94.
- Stuehr DJ, Gross SS, Sakuma I, Levi R, Nathan CF: Activated murine macrophages secrete a metabolite of arginase with the bioactivity of the endothelium-derived relaxing factor and the chemical reactivity of nitric oxide. *J Exp Med* 1989;169:1011-1020.
- Miwa M, Stuehr DJ, Marietta MA, Wishnok JS, Tannen BSR: Nitrosation of amines by Stimulated macrophages. *Carcinogenesis* 1987;8:955-958.
- Jun CD, Ryu H, Um JY, Kim TY, Kim JM, Kang SS, Kim HM, Chung HT: Involvement of protein kinase C in the inhibition of nitric oxide production in murine microglial cells by glucocorticoid. *Biochem Biophys Res Commun* 1994;199:633-638.
- Jun CD, Kim JF, Soh CT, Kang SS, Chung HT: Nitric oxide mediates the toxicoplasmatic activity of murine microglial cells in vitro. *Immunol Invest* 1993;22:487-501.
- Fontana A, Kristensen F, Dubs R, Gerns D, Webew E: Production of prostaglandin and interleukin 1-like factor by cultured astrocytes and C-6 glioma cells. *J Immunol* 1982;129:2413-2419.
- Frei K, Bodmer S, Schwerdel C, Fontana A: An astrocyte-derived interleukin-3 as a growth factor for microglia cells and peritoneal macrophages. *J Immunol* 1986;137:3521-3527.
- Hassan NF, Rifat S, Campbell DE, McCawley LJ, Douglas SD: Isolation and flow cytometric characterization of newborn mouse brain-derived microglia maintained in vitro. *J Leukoc Biol* 1991;50:86-92.
- Langermans JAM, Marielle EB, Hulst VD, Nibbering PH, Furth RV: Activation of mouse peritoneal macrophages during infection with *Salmonella typhimurium* does not result in enhanced intracellular killing. *J Immunol* 1990;144:4340-4346.
- Xie QW, Cho HJ, Calaycay J, Mumford RA, Swiderek KM, Lee TD, Ding A, Troso T, Nathan C: Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* 1992;256:225-228.
- Lyons CR, Orloff GJ, Cunningham JM: Molecular cloning and functional expression of an inducible NOS from a murine macrophage cell line. *J Biol Chem* 1992;267:6370.
- Auffray C, Rougeon F: Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur J Biol* 1980;107:303.
- Jun CD, Choi BM, Ryu H, Um JY, Kwak HJ, Lee BS, Paik SK, Kim HM, Chung HT: Synergistic cooperation between phorbol ester and IFN- γ for induction of nitric oxide synthesis in murine peritoneal macrophages. *J Immunol* 1994;153:3684-3690.
- Nakane M, Mitchell J, Forstermann U, Murad F: Phosphorylation by calcium-calmodulin-dependent protein kinase II and protein kinase C modulate the activity of nitric oxide synthase. *Biochem Biophys Res Commun* 1991;180:1396-1402.
- Brune B, Lapetina EG: Phosphorylation of nitric oxide synthase by protein kinase A. *Biochem Biophys Res Commun* 1991;18:921-926.

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