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Induction of cyclooxygenase-2 by staurosporine through the activation of nuclear factor for IL-6 (NF-IL6) and activator protein 2 (AP2) in an osteoblast-like cell line

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Abstract

The induction of cyclooxygenase-2 (COX-2) plays a crucial role in many physiological and pathological processes. The expression of the *COX-2* gene is regulated by many extracellular stimuli, including growth factors, cytokines, and tumor promoters. Staurosporine, a potential anti-tumor drug, was found recently to up-regulate the expression of the *COX-2* gene in the mouse osteoblast-like cell line MC3T3-E1. The ability of staurosporine to induce the expression of the *COX-2* gene was investigated using luciferase reporters controlled by various *COX-2* core promoter regions. Two *cis*-acting sites for activator protein 2 (AP2) and nuclear factor for IL-6 (NF-IL6), respectively, were identified as responsible for the staurosporine-mediated COX-2 up-regulation. Mutational analysis further verified that both NF-IL6 and AP2 are involved in this process. Further studies showed the stimulatory effect of staurosporine on luciferase activity to be both time- and concentration-dependent. Luciferase activity could be induced at as low as 5 nM staurosporine and reached a maximum at around 20 nM. At 50 nM, the stimulatory effect of staurosporine on luciferase activity reached a maximum at about 8 hr and fell rapidly following 10 hr of incubation. Interestingly, a selective protein kinase C inhibitor, 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF109203X), failed to stimulate luciferase activity under the same conditions. This finding implies that staurosporine-mediated *COX-2* gene expression is specific and independent of protein kinase C activity.

Keywords: Staurosporine; NF-IL6; AP2; Cyclooxygenase-2

1. Introduction

Staurosporine, a microbial antifungal alkaloid of *Streptomyces staurospores*, potently inhibits serine/threonine protein kinases [1,2]. Although staurosporine is widely used to explore the physiological functions of many protein kinases, it may participate in inducing cellular responses independent of its protein kinase inhibitory activity, as implied by several reports. For example, staurosporine can cause the differentiation of keratinocytes, as described by Jones and Sharpe [3]. A selective PKC inhibitor, Ro 31-8220, did not mimic the effect of staurosporine. In rat neuroblastoma PC-12 cells, staurosporine was demonstrated to activate neurite outgrowth and differentiation by activating c-Jun N-terminal kinase [4]. In rat fibroblasts, however, the expression of collagenase was enhanced by a nanomolar range of staurosporine concentrations independently of PKC [5]. Staurosporine also acted synergistically with cyclic AMP (cAMP) in a neuroblastoma cell line, NB-OK-1, to stimulate a progressive increase in the protein level of VGF, a neural specific protein, and that of tyrosine hydroxylase [6]. The expressions of nitric oxide synthase (NOS) and interleukin-2 were actively promoted by staurosporine in vascular smooth muscle cells [7]. In contrast, neither of the potent PKC inhibitors, calphostin C or Ro 31-8820, was able to induce a similar effect. The up-regulation

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Abbreviations: AP2, activator protein 2; COX, cyclooxygenase; DDAB, dimethyldioctadecylammonium bromide; DOPE, dioleoyl-L-α-phosphatidylethanolamine; NF-IL6, nuclear fsactor for IL-6; PCR, polymerase chain reaction; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; $pPLA_2$, porcine phospholipase A_2.

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of interleukin expression by staurosporine has also been observed in human HL-60 cells [8], EL4 thymoma cells [9], and human keratinocytes [10].

Staurosporine was observed recently to induce the expression of COX-2 (EC 1.14.99.1) in rat macrophages [11,12]. Preliminary results from our laboratory also suggest that staurosporine can induce the *COX-2* gene in a murine osteoblast-like cell line, MC3T3-E1. Cyclooxygenase is the key enzyme in the conversion of arachidonic acid to prostanoids and thromboxanes [13–15]. Two distinct COX isozymes, COX-1 and COX-2, have been identified and cloned in many species. COX-1 is generally considered to be a constitutive enzyme present in a variety of tissues and is thought to be involved in housekeeping functions. COX-2, however, is rapidly and transiently induced by growth factors, cytokines, and tumor promoters [16–19], and is selectively inhibited by glucocorticoids. COX-2 may play essential roles in several biological functions, including inflammatory responses and tumorigenesis. Although the regulation of the *COX-2* gene by various agonists has been studied extensively, the molecular mechanism underlying staurosporine-induced *COX-2* expression remains unknown.

The sequence of the *COX-2* promoter is known, and multiple putative *cis*-acting elements for transcription factors, such as NF-κB, NF-IL6, AP2, ATF/CRE, and E-box, have been identified [20–22]. The consensus *cis*-acting sites of NF-κB, NF-IL6, and CRE are major factors in responding to the stimulation of hormones, cytokines, and tumor promoters. The present study demonstrates that the potent protein kinase inhibitor staurosporine significantly induces COX-2 production in MC3T3-E1 cells. Interestingly, this induction occurs by activating AP2 and NF-IL6 in a PKC-independent manner.

2. Materials and methods

2.1. Materials

Alpha-modified Eagle' minimum essential medium (AMEM), trypsin/EDTA, penicillin G/streptomycin, fetal bovine serum (FBS), and TRIzol were from Life Technology. Staurosporine and 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF109203X) were products of Calbiochem. $pPLA_2$-IB was purchased from Boehringer Mannheim. Anti-mouse COX-2 antibody and horseradish peroxidase-conjugated secondary antibody were acquired from Santa Cruz Biotechnology. *Pfu* DNA polymerase, PCR buffer and other reagents for PCR, and a QuikChange Site-Directed Mutagenesis kit were bought from Strategene. DOPE, DDAB, and 3,3-diaminobenzidine tetrahydrochloride (DAB) were obtained from the Sigma Chemical Co. Oligonucleotide primers for PCR were custom synthesized by BioBasic Inc. All other reagents were reagent grade.

2.2. Cell culture

MC3T3-E1 cells, provided by Dr. Barid Mukherjee, McGill University, were maintained in 75-cm^2 culture flasks with 10 mL AMEM containing 10% FBS, 100 units/mL of penicillin G, and 100 μg/mL of streptomycin. The cells were grown at 37° in a humidified incubator containing 5% CO_2. Cells were subcultured every 2–3 days.

2.3. Construction of luciferase reporter vectors

Luciferase reporter vectors, pGLB/18-NA (CRE site removed) and pGLB/18-N (with NF-IL6 site only), were generated from pGLB/18 (containing a 5′-flanking region −188 to +71 of the mouse *COX-2* gene) and pGLB/mu-AP2 [19], respectively, by mutating the CRE consensus site using a QuickChange site-directed mutagenesis kit according to the instructions of the manufacturer. A primer pair was designed for CRE site mutagenesis: 5′-TCACCAC-TACGCTATGTGGAGTCCGC-3′ and 5′-GCGGACTC-CACATAGCGTAGTGGTGA-3′.

2.4. Transfection of MC3T3-E1 cells with plasmids and the luciferase assay

DOPE:DDAB (2:1 molar ratio) liposomes were prepared as described by Campbell [23]. MC3T3-E1 cells (3×10^4/well) were cultured in 12-well culture plates to about 60% confluence before transfection. To each well, 0.7 μg luciferase vector and 8.4 μL liposome in 1 mL Opti-MEM (Life Technologies, Inc.) were added and incubated at 37° for 6 hr. The cells were then grown to nearly 100% confluence. Subsequently, cells were serum starved with AMEM containing 0.1% bovine serum albumin 18–24 hr prior to the treatment. After treatment, cells were harvested, and luciferase activity of the cell lysates was determined on a 1254 LUMINOVA Luminometer (BioOrbit). The luciferase activity was normalized based on the protein concentration of each cell lysate.

2.5. Northern blotting

Northern blotting was done as described previously [19]. Total RNA was isolated from MC3T3-E1 cells by using TRIzol reagent according to the instructions of the manufacturer. Thirty micrograms of total RNA was resolved by electrophoresis on a formaldehyde-containing 1.2% agarose gel and transferred onto Hybond-N + nylon membrane. The cDNAs corresponding to nucleotide sequence 2244-3121 (878 bp in length) of the reported mouse *COX-2* gene [24] and to nucleotide sequence 139–858 (720 bp in length) of the mouse glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene [25] were used as probes. Both DNA probes were labeled with [α-^{32}P]dCTP using a random primer labeling kit (Life Technologies). After

denaturation, the labeled probes were hybridized with the RNA blots for 2 hr at 68° using ExpressHyb solution (Clontech). Specific bands hybridizing with the probes were detected by autoradiography. The amount and the quality of the RNA loaded were monitored by hybridization with the GAPDH.

2.6. Western blotting

MC3T3-E1 cells were washed once with PBS and collected by scraping with a rubber policeman. Cell lysates were prepared by incubating cell pellets with the cell lysis buffer [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.2% deoxycholate] containing 1 mM phenylmethylsulfonyl fluoride (PMSF) on ice for 15 min. Equal amounts (25 μg) of cell lysate proteins were than resolved on a 10% SDS-polyacrylamide gel. Subsequently, protein bands were transferred to an Immobilon-P polyvinylidene fluoride (PVDF) membrane (Millipore) and blocked with 5% non-fat milk in PBS at room temperature for 2 hr. The membrane was then incubated with antibody against COX-2 (1:500) followed by a horseradish peroxidase-conjugated secondary antibody against rabbit IgG (1:5000). The COX-2 protein band was then visualized by DAB (5 mg/mL).

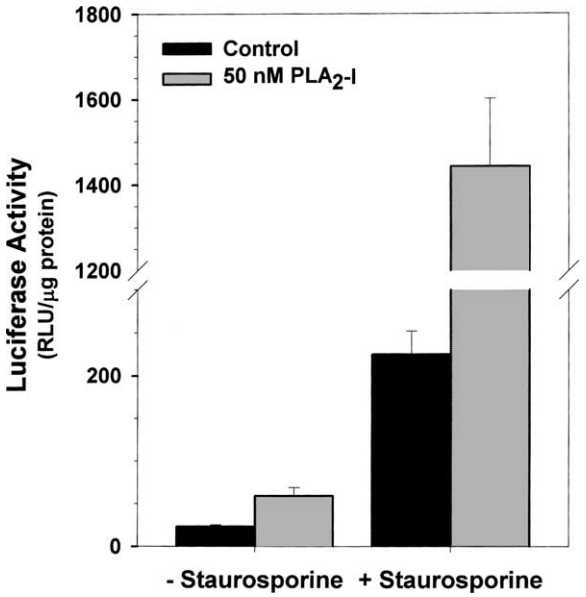

Fig. 1. Effect of staurosporine on $pPLA_2$-IB-mediated COX-2 induction in MC3T3-E1 cells. Luciferase reporter vector pGLB/18-transfected MC3T3-E1 cells were pretreated with 50 nM staurosporine at 37 ° for 1 hr. After pretreatment, buffer (black columns) or 50 nM $pPLA_2$-IB (gray columns) was added, and the cells were incubated further at 37 ° for another 8 hr. Then luciferase activity was determined. Data shown are the means ±SD for three separate experiments, each carried out in triplicate. RLU = random light unit.

3. Results

3.1. Stimulation of COX-2 synthesis in MC3T3-E1 cells by staurosporine

Previous studies have demonstrated that porcine phospholipase A_2-I ($pPLA_2$-IB) can increase both mRNA and protein levels of COX-2 in MC3T3-E1 cells [19]. Staurosporine was found to be able to facilitate the stimulatory effect of $pPLA_2$-IB (Fig. 1) in the signaling pathway of $pPLA_2$-IB-induced *COX-2* gene up-regulation. The luciferase activity of pGLB/18-transfected cells, upon treatment with 50 nM staurosporine, was 10 times (without 50 nM $pPLA_2$-IB) and 24 times (with 50 nM $pPLA_2$-IB) higher than that of the control cells, respectively. This result suggested that staurosporine, alone, not only promoted the stimulatory effect of $pPLA_2$-IB but also induced the expression of the *COX-2* gene. Northern and western blotting analyses confirmed this observation (Fig. 2). As shown in Fig. 2A, the mRNA level of the *COX-2* gene markedly increased in MC3T3-E1 cells 30 min following treatment with 50 nM $pPLA_2$-IB (lane 2), 50 nM staurosporine (lane 3), or both (lane 4). The amount of mRNA, however, was increased greatly in the presence of both staurosporine and $pPLA_2$-IB. A similar result was observed in the intracellular protein level of COX-2 before and after treatment (Fig. 2B). These findings clearly indicate that staurosporine, alone, induced the expression of the *COX-2* gene in MC3T3-E1 cells. In contrast, staurosporine did not induce *COX-1* in MC3T3-E1 cells (data not shown).

3.2. Induction of luciferase activity by staurosporine but not by GF109203X

Although the stimulatory effect of staurosporine on *COX-2* gene expression has been demonstrated, the molecular mechanism underlying this process has not been elucidated. PKC was first hypothesized as possibly participating in staurosporine-induced *COX-2* gene expression, since staurosporine was shown actively to regulate the kinase activity of PKC in numerous studies. Therefore, a more selective PKC inhibitor, GF109203X, instead of staurosporine was employed to treat pGLB/18-transfected MC3T3-E1 cells. As shown in Fig. 3A, the luciferase activity of pGLB/18-transfected cells remained low, under treatment with various amounts of GF109203X (0–500 nM). Similarly, no significant effect was observed on staurosporine-mediated luciferase activity induction in pGLB/18-transfected cells with GF109203X at concentrations up to 200 nM (Fig. 3B). These results indicate that PKCs did not participate in staurosporine-mediated *COX-2* gene up-regulation.

3.3. Time- and concentration-dependent up-regulation of COX-2 by staurosporine

As shown in Fig. 4B, 50 nM staurosporine induced luciferase activity in pGLB/18-transfected cells in a time-dependent manner. Induction of luciferase activity

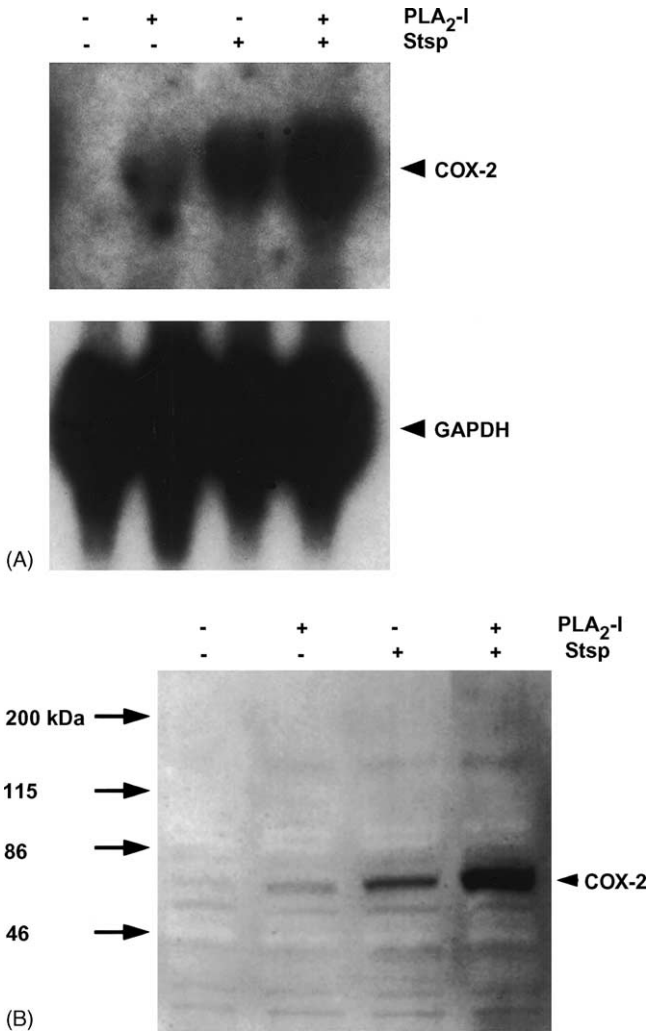

Fig. 2. Induction of *COX-2* gene expression by staurosporine. (A) Northern analysis of total RNA from MC3T3-E1 cells probed for COX-2 and GAPDH. Total RNA was prepared from mock-treated MC3T3-E1 cells (lane 1), or cells treated with 50 nM $pPLA_2$-IB (lane 2), 50 nM staurosporine (lane 3), or both 50 nM $pPLA_2$-IB and 50 nM staurosporine (lane 4) at 37° for 30 min. (B) Western analysis of whole cell lysate of MC3T3-E1 cells. Cell lysate was obtained from cells incubated with 50 nM $pPLA_2$-IB, 50 nM staurosporine, or both at 37° for 6 hr. Cell lysate protein (25 μg) was separated using 10% SDS–PAGE followed by western blot analysis.

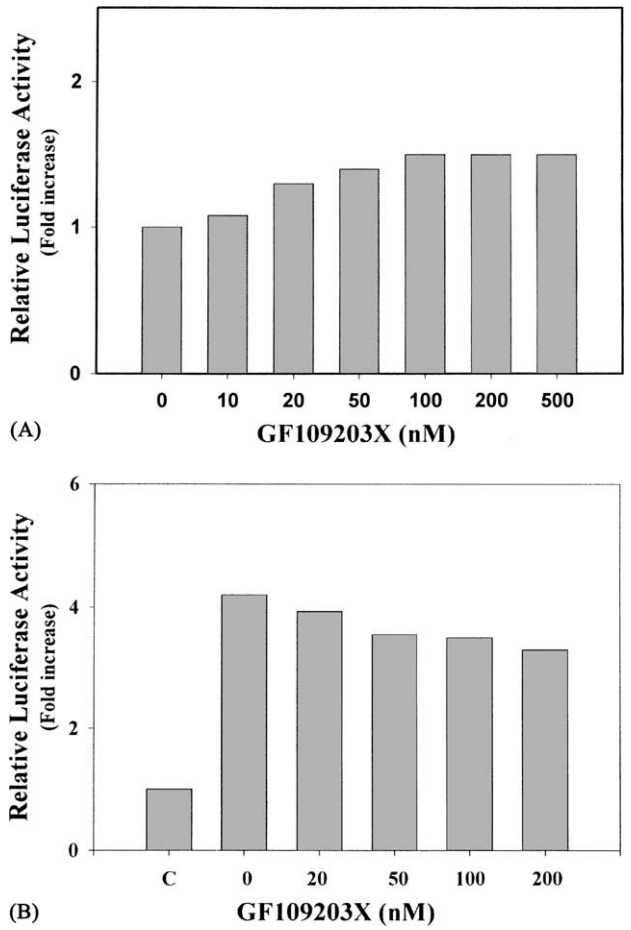

Fig. 3. Effect of the selective PKC inhibitor GF109203X on luciferase reporters controlled by the *COX-2* promoter. (A) The pGLB/18-transfected MC3T3-E1 cells were treated with various amounts of GF109203X (0–500 nM) at 37° for 8 hr. Luciferase activity was then determined and analyzed. (B) The pGLB/18-transfected MC3T3-E1 cells were treated with 20 nM staurosporine in the presence of various amounts of GF109203X (0–200 nM) at 37° for 8 hr. (C represents control cells transfected with pGL-Basic vector only.) The results shown are the means of two separate experiments, each carried out in triplicate.

was first observed 4 hr following the addition of staurosporine. The activity reached a maximum 8 hr after incubation. Thereafter, the stimulatory effect of staurosporine declined rapidly, and luciferase activity returned to basal levels at 24 hr. The pGLB/18-transfected cells exhibited gradually increasing luciferase activity (Fig. 4A) when incubated with various concentrations of staurosporine (5–200 nM). At 20 nM staurosporine, luciferase activity was about fifteen times higher than that of the control. The stimulatory effect, however, declined rapidly when the concentration of staurosporine in the medium was at least 100 nM. These results suggest that staurosporine induces a transient, concentration-dependent activation of the expression of the *COX-2* gene.

3.4. Analysis of the promoter region of the COX-2 gene

Previous work has shown that the 5′-flanking region of nucleotide sequences −188 to +71 of the *COX-2* gene contains *cis*-responding elements, which can be activated by staurosporine. The 5′-flanking region between −639 and +71 of the *COX-2* gene was analyzed by series deletion to rule out the involvement of other transcription factors in the activation by staurosporine. As depicted in the left panel of Fig. 5, a variety of putative responding elements, including MEF-2, NF-κB, SP1, AP2, NF-IL6, CRE, E-box, and TATA box, have been identified in this region [20–22]. The luciferase vectors containing promoter regions that cover various lengths of the *COX-2* gene were obtained [19]. Each vector was transfected to MC3T3-E1 cells by lipofection. Cells were harvested, and luciferase activity of the cell lysate was measured 6 hr following treatment with 20 nM staurosporine. As shown in the right

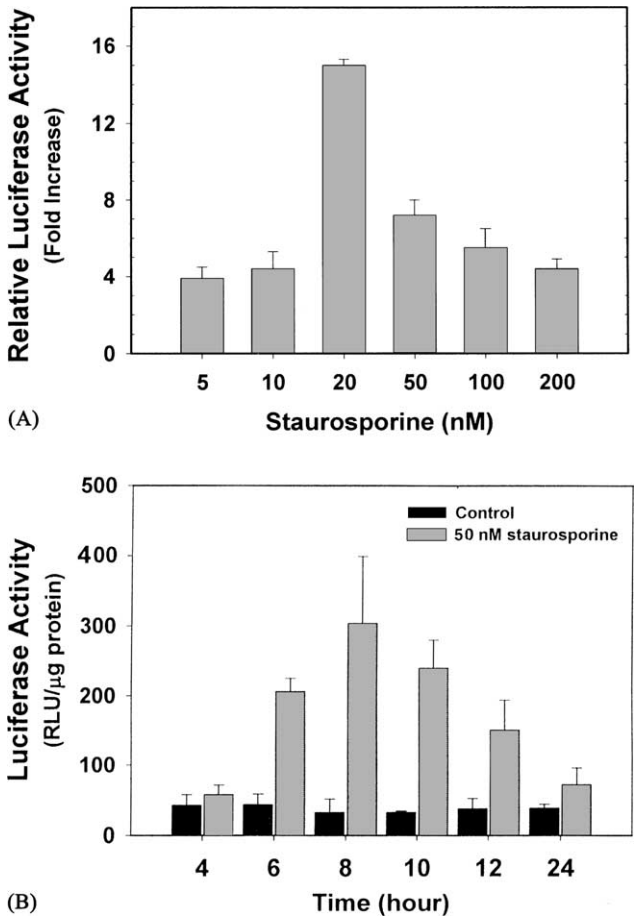

Fig. 4. Effect of staurosporine on the stimulation of *COX-2* promoter in MC3T3-E1 cells. (A) The staurosporine concentration response on the luciferase activity of pGLB/18-W-transfected cells. The pGLB/18-W-transfected cells were incubated with various amounts (5–200 nM) of staurosporine at 37° for 8 hr. After incubation, cells were harvested, and cell lysates were subjected to activity assay. (B) Time-course of the induction of luciferase activity in pGLB/18-W-transfected cells. The luciferase plasmid pGLB/18-W-transfected cells (1×10^5) were incubated with 50 nM staurosporine. At the indicated time intervals, the cells were harvested and subjected to a luciferase activity assay. Results are the means ±SD for three separate experiments, each carried out in triplicate. RLU = random light unit.

panel of Fig. 5, staurosporine induced significant luciferase activity in cells transfected with pGLB/60 (−639 to +71), pGLB/40 (−478 to +71), pGLB/20 (−283 to +71), and pGLB/18 (−188 to +71). The luciferase activity decreased markedly when the region between nucleotide sequences −188 and −133 was deleted. Interestingly, this region contains two *cis*-responding elements, NF-IL6 (−138 to −130) and AP2 (−150 to −142). This result was consistent with previous observations and suggested that both transcription factors NF-IL6 and AP2 may be activated by staurosporine.

3.5. Mutational analysis of the promoter region of the COX-2 gene

The region between −188 and −133 contains responding elements for transcription factors NF-IL6 and AP2. Five luciferase reporter vectors, pGLB/18, pGLB/mu-AP2 (containing NF-IL6 and CRE sites), pGLB/mu-C/EBPβ (containing AP2 and CRE sites), pGLB/18-NA (containing NF-IL6 and AP2 sites), and pGLB/18-N (containing NF-IL6 only), were obtained either from previous work [19] or through site-directed mutagenesis to determine which transcription factors may be involved in inducing the *COX-2* gene with staurosporine. Each vector was transfected into MC3T3-E1 cells, and the luciferase activities of these cells before and after staurosporine treatment were measured and compared. As shown in Fig. 6, significant luciferase activity of all transformed cell lines was induced by 20 nM staurosporine to a level similar to that of the wild-type reporter plasmid, pGLB/18. These results suggest that NF-IL6 and AP2 worked either alone or together in the staurosporine-induced *COX-2* gene expression.

4. Discussion

The authors' previous studies have demonstrated that porcine phospholipase A_2-I (pPLA_2-IB), a family of acyl esterases that catalyze the hydrolysis of phospholipids, could induce *COX-2* gene expression in MC3T3-E1 cells [19] via its cell surface receptor. A specific transcription factor, NF-IL6 or C/EBPβ, was activated during this process. This study further demonstrated that staurosporine can facilitate pPLA_2-IB-mediated *COX-2* gene induction in MC3T3-E1 cells (Figs. 1 and 2). Interestingly, staurosporine alone can also stimulate the expression of the *COX-2* gene in MC3T3-E1 cells. Northern and western blot analyses (Fig. 2) further verified the stimulatory effect of staurosporine. The induction of the *de novo* biosynthesis of COX-2 by staurosporine in the rat macrophage has also been reported [11,12]. The molecular mechanism underlying the staurosporine-mediated process in rat macrophages, however, is not known. The microbial alkaloid staurosporine, originally identified as a potent inhibitor of serine/threonine protein kinases, has been shown to stimulate a broad spectrum of cellular events, including cell cycle arrest [26,27], apoptosis [28–30], neuronal differentiation [3,4], and gene regulation [5–12]. The mechanism responsible for these cellular events is not known, but staurosporine-mediated cellular processes are believed to be independent of its inhibitory activity.

Staurosporine was shown to induce the expression of nitric oxide synthase and interleukins by activating NF-κB as described in many reports [7–10]. C/EBP family members may also be involved in staurosporine-mediated processes, as postulated by Hecker et al. [7]. Notably, the 5′-flanking region of the mouse *COX-2* gene also contains consensus sites for NF-κB (−401 to −393) and NF-IL6 (−138 to −130) [20,21]. The 5′-flanking region (−639 to +71) of the *COX-2* gene was analyzed by series deletion to further elucidate the molecular mechanism of staurosporine-mediated gene up-regulation in MC3T3-E1 cells. The

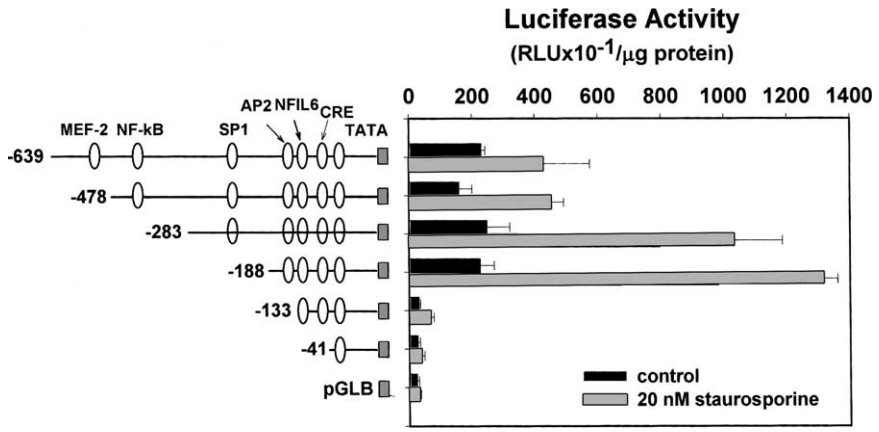

Fig. 5. Deletion analysis of the *COX-2* gene promoter region. Putative consensus *cis*-responding sequences in the 5′-flank region of the mouse *COX-2* gene are illustrated on the left. Each deleted promoter fragment was inserted into a luciferase plasmid, pGL-Basic (Promega). Numbers indicate distances in base pairs from the start of transcription. Each of these reporter vectors was transfected into MC3T3-E1 cells using the lipofection method as described under Section 2. Cells were cultured for another 24 hr and then incubated without (black columns) or with (gray columns) 20 nM staurosporine at 37° for 6 hr. Luciferase activity was assayed, and the results were normalized with the protein concentration. Data shown are the means ±SD for three separate experiments, each carried out in triplicate. RLU = random light unit.

minimal region of the *COX-2* gene promoter found to be essential for staurosporine-mediated gene expression was between −188 and −133, from the beginning of the first exon (Fig. 5). This finding eliminated the involvement of NF-κB and CRE (−56 to −51) in the induction of the *COX-2* gene by staurosporine in MC3T3-E1 cells. As shown above, the region −188 to −133 contains two *cis*-responding sites for transcription factors NF-IL6 and AP2. Site-directed mutagenesis of the promoter region (−188 to +71) was conducted to further examine the possible transcription factor(s) that might be involved in staurosporine-mediated *COX-2* expression. Both NF-IL6 and AP2 were demonstrated to be activated by staurosporine in MC3T3-E1 cells (Fig. 6) when the reporter plasmid contained a single consensus site for NF-IL6 or AP2.

NF-IL6, a member of the CCAAT/enhancer binding protein (C/EBP) family of transcription factors, is involved in inducing several acute-phase protein genes in response to immune and inflammatory stimulation. NF-IL6 also plays a major role in inducing the expression of *COX-2* by hormones, cytokines, endotoxin, and tumor promoters [18,21,31–33] Transcription factor AP2, however, is in a class of enhancer-binding proteins that can be activated during vertebrate embryogenesis and acute inflammation. Much research has suggested that the activity of both NF-IL6 and AP2 can be regulated by phosphorylation [34–38]. AP2 can be activated by the phosphorylation of PKA and PKC [34,35], while NF-IL6 can be phosphorylated and activated by PKA [36], PKC [36,37], mitogen-activated protein kinase (MAPK) [38], and calmodulin-dependent protein kinase-II [39]. Clearly, protein kinases, especially PKA and PKC, are central to regulating transcription factors NF-IL6 and AP2.

Although staurosporine effectively inhibits PKC activity, it was reported recently to exhibit PKC-agonistic

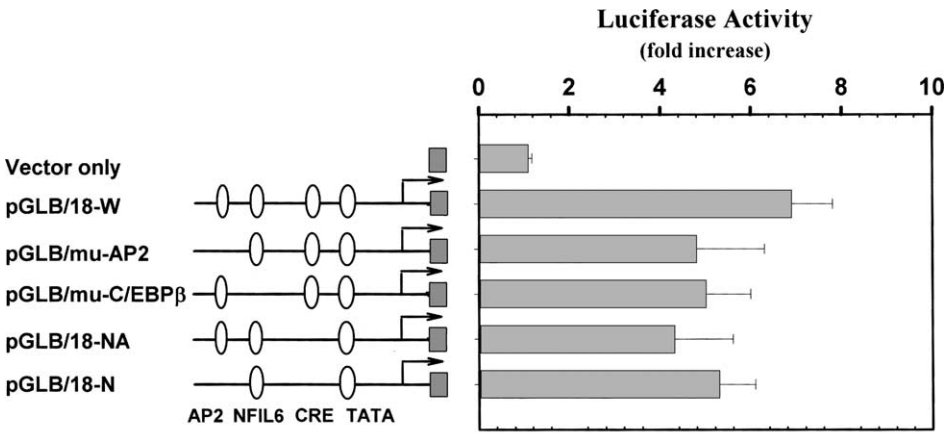

Fig. 6. Mutational analysis of the 5′-flanking region (−188 to +71) of *COX-2*. The left panel represents the constructed reporter vectors containing wild-type and mutated promoter regions of the *COX-2* gene. Each plasmid was transfected into MC3T3-E1 cells by lipofection. The cells were then incubated with 20 nM staurosporine at 37° for 6 hr. Luciferase activity was assayed, and the results were normalized with protein concentration. Data shown are the means ±SD for three separate experiments, each carried out in triplicate.

activity and to induce cellular redistribution and activation of several PKC isoforms, including PKC-α, -θ, -γ, and -ϵ [40–42]. Accordingly, the role of PKCs in staurosporine-mediated *COX-2* expression in MC3T3-E1 cells was examined. A more selective PKC inhibitor, GF109203X, was used instead of staurosporine, to clarify the involvement of PKC in staurosporine-induced *COX-2* gene expression. GF109203X, an analog of staurosporine, competes with ATP as a competitive inhibitor and shows high selectivity for PKC ($K_i = 10$ nM) [43]. As shown in Fig. 3A, GF109203X (10–500 nM) alone could not induce *COX-2* gene expression in MC3T3-E1 cells. Furthermore, GF109203X at 200 nM did not affect significantly the stimulatory effect of staurosporine on the luciferase activity of pGLB/18-transfected cells (Fig. 3B). Apparently, PKCs did not participate in staurosporine-mediated signaling in MC3T3-E1 cells. Other protein kinase inhibitors (for example, H-89, the specific inhibitor of protein kinase A; hypericin, the inhibitor of casein kinase II; and KT-5926, the inhibitor of myosin light chain kinase) were also ineffective (data not shown). These observations clearly show that the stimulatory effect of staurosporine on the *COX-2* gene is specific and independent of its protein kinase inhibitory activity.

The MEKK1/JNK signaling pathway was demonstrated recently to participate in hormone- and endotoxin-induced *COX-2* gene expression in MC3T3-E1 osteoblast-like cells and in RAW 264.7 macrophages [32,33]. Interestingly, staurosporine was observed to activate a novel JNK isoform and induce neurite outgrowth of the rat neuroblastoma cell line PC-12 [4]. Although the mechanism by which staurosporine activates this novel JNK isoform in PC-12 cells is unknown, staurosporine is postulated to do the same in MC3T3-E1 cells by signaling through the MEKK1/JNK pathway to activate AP2 and NF-IL6. In conclusion, staurosporine has been shown for the first time to possibly induce the expression of the *COX-2* gene by activating transcription factors, NF-IL6 and AP2, in MC3T3-E1 cells. The signaling pathway of staurosporine, leading to the activation of both NF-IL6 and AP2, however, is still not understood.

Acknowledgments

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