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REGULATION OF NUR77 GENE EXPRESSION BY PROSTANOIDS IN CEMENTOBLASTIC CELLS

Sanda M. Moldovan, DDS, MS^{*}, Jeanne M. Nervina, DMD, PhD[†], Sotirios Tetradis, DDS, PhD[‡], and Paulo M. Camargo, DDS, MS[§]

^{*}Formerly of the Division of Associated Clinical Specialties, Section of Periodontics, School of Dentistry, University of California, Los Angeles Currently in Private Practice

[†]Division of Associated Clinical Specialties, Section of Orthodontics, School of Dentistry, University of California, Los Angeles

[‡]Division of Surgical and Diagnostic Sciences, Section of Oral Radiology, School of Dentistry, University of California, Los Angeles

[§]Division of Associated Clinical Specialties, Section of Periodontics, School of Dentistry, University of California, Los Angeles

Abstract

Objective—The inflammatory cytokine interleukin-1 (IL-1) decreases mineralization by immortalized mouse-derived cementoblastic cells (OC-CM cells), while various prostanoids, including fluprostenol (flup) increase it. Subtraction hybridization conducted on flup- minus IL-1-treated OC-CM cells revealed that one of the primary response genes preferentially induced by flup is the transcription factor Nur77. The objective of this study was to examine the signal transduction cascades regulating prostanoid induction of Nur77 gene expression in OC-CM cells.

Methods—Confluent OC-CM cells were treated with prostaglandin E₂ (PGE₂), prostaglandin F_{2α} (PGF_{2α}), specific activators of the various EP prostanoid receptors and of the FP prostanoid receptor, and direct activators/inhibitors of the cyclic AMP-protein kinase A (PKA), protein kinase C (PKC) and intracellular calcium pathways. Nur77 gene expression was examined by mRNA extraction and northern blot analysis.

Results—PGE₂ and PGF_{2α} treatment of OC-CM cells significantly increased Nur77 mRNA expression in a time- and dose-dependent fashion. Both the EP1 prostanoid receptor-specific activator 16, 16 dimethyl PGE₂ and the FP prostanoid receptor-specific activator flup significantly increased Nur77 gene expression by OC-CM cells as compared to vehicle-treated controls. Increase in Nur77 gene expression was also observed when direct activators of the PKA, PKC and intracellular calcium pathways were used to treat OC-CM cells. Direct inhibition of the PKA, PKC and intracellular calcium pathways abrogated Nur77 gene expression induced by OC-CM cell treatment with PGE₂ and PGF_{2α}.

Corresponding Author: Dr. Paulo M. Camargo, UCLA School of Dentistry, 10833 Le Conte Ave., Periodontics CHS 63048, Los Angeles, CA 90095, Phone: (310) 825 0928, Fax: (310) 206 3282, E-mail: pcamargo@dentistry.ucla.edu.

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Conclusion—Nur77 is a primary gene expressed by OC-CM cells and its induction appears to be mediated by the PKA, PKC and intracellular calcium pathways. Nur77 may affect expression of downstream target genes in OC-CM cells and partially regulate cementoblast cell function.

Keywords

Cementum; cementogenesis; periodontal regeneration; prostaglandin

INTRODUCTION

Periodontitis is an inflammatory condition of the supporting structures of the teeth and is characterized by clinical attachment loss and alveolar bone destruction (1). As periodontitis progresses periodontal pocket depth increases and that contributes to the perpetuation of the disease process (2). Deep pockets favor plaque accumulation, which results in more inflammation and tissue destruction (3). An important goal in the treatment of periodontitis is the reduction of periodontal pockets to a physiologic depth, which will lead to the creation of an environment that facilitates plaque control (4). Ideal pocket reduction involves the regeneration of bone, periodontal ligament (PDL) fibers and cementum (5). However, *de novo* formation of cementum in the periodontal regenerative process is difficult and often incomplete (6). Therefore, understanding cementoblast biology and the cellular and molecular events associated with cementogenesis may lead to therapeutic strategies aimed at predictable periodontal regeneration.

Interleukin-1 (IL-1) and prostaglandins (PGs) are inflammatory mediators that have been associated with the initiation and progression of periodontitis (7). Paradoxically, the effects of IL-1 and PGs on osteoblastic function are distinctly different. While IL-1 has a catabolic effect on bone (8-10), prostaglandin E₂ (PGE₂) has a biphasic effect on osteoblast function (11-14). Depending on the dosage and mode of administration, PGE₂ can either promote or inhibit the formation of bone (15-17).

The effects of IL-1 and PGs on cementum are less well understood than those on bone. Locally delivered PGE₁ increases the formation of alveolar bone, PDL and cementum in adult dogs (18). IL-1 inhibits mineralization by an immortalized cementoblastic cell line (OC-CM cells) while PGE₂ and PGF_{2α} have an anabolic effect on mineral nodule formation by OC-CM cells (19). Fluprostenol (flup), a synthetic prostanoid agent with a molecular structure similar to PGF_{2α}, exerts a well-defined, dose-dependent anabolic effect on mineralization by OC-CM cells (19). While this anabolic effect appears to be, in part, mediated by activation of the protein kinase C (PKC) signaling pathway (19), the exact molecular mechanism of the prostanoid-induced mineralization by OC-CM cells is not well understood.

The effects of prostanoids on cells are mediated through G protein-coupled prostaglandin receptors (20). PGE₂ activates four EP receptor subtypes (EP1-4), while PGF_{2α}, and the synthetic PGF_{2α} analog flup, activate FP receptors. The EP1 and FP receptors preferentially activate the PKC signaling pathway and calcium signaling. EP2 and EP4 receptors activate, while EP3 receptors inhibit, the cyclic AMP-protein kinase A (PKA) signaling pathway (19, 21).

In an attempt to elucidate the mechanism by which prostanoids increase mineralization by cementoblastic cells, cDNA subtraction hybridization of OC-CM cells treated with flup minus those treated with IL-1 was conducted in presence of the protein synthesis inhibitor cycloheximide (22). The subtraction hybridization revealed several primary response genes that were preferentially induced by flup, which may therefore be involved in the regulation of cementoblast function (22). One of the preferentially flup-induced genes in OC-CM cells is

Nur77, an NGFI-B nuclear orphan receptor whose role as a transcription factor is well documented (23,24). From these findings, it could be hypothesized that induction of the Nur77 gene affects expression of downstream target genes in OC-CM cells and partially regulates cementoblast function. The purpose of this study was to examine the signaling cascades that mediate prostanoid regulation of Nur77 gene expression in OC-CM cells.

MATERIALS AND METHODS

Materials

PGE₂, PGF_{2α}, phorbol 12-myristate 13-acetate (PMA), forskolin (FSK), ionomycin, H89 and 1,2-Bis(2-aminophenoxy)ethane-N,N',N'-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA) were purchased from Sigma (Saint Louis, MO); 16,16-Dimethyl-prostaglandin E₂ (16,16-DM-PGE₂) from Biomol (Plymouth Meeting, PA); and butaprost, sulprostone, misoprostol, and flup from Cayman (Ann Arbor, MI).

Cell culture

OC-CM cells (a gift from Dr. Martha J. Somerman, University of Washington, Seattle) were grown on 10 cm tissue culture dishes (Corning, Corning, NY) in DMEM (Gibco, Grand Rapids, MI) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), 100 mU/ml penicillin and 100 µg/ml streptomycin (Pen/Strep, Gibco) at 37°C in 5% CO₂ and were passaged at 30,000 cells/ml. Cells between passages 14 and 19 were used for experiments. Upon confluence, the culture medium was changed and cell treatment with various agents was initiated. Cell treatment was performed for 30 minutes up to 8 hours in various experiments.

RNA isolation and northern blot analysis

Total RNA was isolated by a single-step method using Trizol (Invitrogen, Carlsbad, CA). Twelve micrograms of RNA were fractionated by electrophoresis on a 1% agarose gel containing 9% formaldehyde for 2.5 hours. RNA was transferred overnight to a Gene Screen Plus hybridization membrane (NEN Life Science Products, Boston, MA) and exposed to UV using a SpectroLinker XL-100 (Spectronics Corporation, Westbury, NY). Radioactive cDNA probes were made using Nur77 and GAPDH cDNA, random primers, DNA polymerase, α³²P-radiolabelled cytosine, and a mixture of thymine, guanine, and adenine. Membranes were hybridized using QuikHyb hybridization solution (Stratagene, La Jolla, CA) for 1 hour at 68°C in the presence of 1×10⁷ to 2×10⁷ counts of ³²P-labeled probe.

Membranes were washed twice in 2 × SSC and 0.1% SDS for 15 minutes at room temperature and once in 0.1 × SSC and 0.1% SDS for 15 minutes at 55°C. Membranes were used to expose radiographic films at -80°C using an intensifying screen.

Phosphoimaging

Membranes were exposed to a phospho-imager screen (Fisher Scientific, Pittsburgh, PA) at room temperature overnight. Band intensity was assessed with the use of a Storm 840 PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and ImageQuant software (General Electric Healthcare Bio-Sciences Corporation, Piscataway, NJ).

Data analysis and statistics

All experiments were performed at least in triplicate. Sample means and standard errors were calculated utilizing Microsoft Excel (Redmond, WA). The independent variables for all experiments were treatments administered to OC-CM cell cultures. The dependent variable was the mRNA gene expression of Nur77. The outcome variable was the ratio of Nur77 to GAPDH mRNA as determined by ImageQuant. Parametric statistics were used, as the outcome

was quantitative and continuous. T-tests and two-way ANOVA were used to compare means of the three experiments across treatment groups. T-tests were performed using Excel. ANOVA was performed using the Prism software (GraphPad, San Diego, CA), with post-test repeated measures analyzed by the Bonferroni procedure. P-values ≤ 0.05 were considered significant.

RESULTS

PGE₂ and PGF_{2 α} increased Nur77 mRNA gene expression by OC-CM cells

The expression of Nur77 in the mRNA of OC-CM cells treated with PGE₂ and PGF_{2 α} occurred in a time- and dose-dependent fashion. Nur77 mRNA gene expression by OC-CM cells treated with PGE₂ and PGF_{2 α} was significantly increased at 30 minutes after treatment, peaked at 1 hour, and returned to baseline levels by 4 hours (figure 1). Maximum expression of Nur77 in mRNA was observed when OC-CM cells were treated for 1 hour with PGE₂ at a concentration of 10⁻⁵M, with a significant effect occurring at a concentration as low as 10⁻⁸M (figure 2). Similarly, PGF_{2 α} treatment of OC-CM cells resulted in maximum expression of Nur77 in mRNA of OC-CM cells at a concentration of 10⁻⁵M, with a significant effect observed at a concentration as low as 10⁻⁹M (figure 2).

Effect of selective EP and FP prostanoid receptor agonists on Nur77 mRNA gene expression by OC-CM cells

PGE₂ and PGF_{2 α} signal through activation of EP and FP prostanoid receptors. In order to examine the role played by the four EP prostanoid receptors as well as by the FP prostanoid receptor on the increased expression of Nur77 in mRNA of cementoblastic cells treated with PGE₂ and PGF_{2 α} , OC-CM cells were treated with various EP and FP prostanoid receptor activators (figure 3). Selective activators of the EP2 (butaprost), EP3 (sulprostone) and of the EP2, EP3 and EP4 (misoprostol) prostanoid receptors did not affect Nur77 mRNA gene expression by OC-CM cells. However, the selective EP1 prostanoid receptor agonist 16,16-DM-PGE₂ and the selective FP prostanoid receptor agonist flup significantly increased Nur77 mRNA gene expression by OC-CM cells as compared to vehicle-treated controls.

The effect of direct intracellular signaling pathway activators and inhibitors on Nur77 mRNA gene expression by OC-CM cells

PGE₂ and PGF_{2 α} , by virtue of binding to EP and FP prostanoid receptors, can activate both the PKA and the PKC signaling pathways and can also regulate intracellular calcium (20). As an attempt to dissect the intracellular pathway(s) involved in the modulation of Nur77 mRNA gene expression by OC-CM cells treated with PGE₂ and PGF_{2 α} , cells were treated with direct activators/inhibitors of the PKA and PKC signaling pathways and with a direct regulator of intracellular calcium. FSK (a direct activator of the PKA pathway) at a concentration of 10⁻⁵M, PMA (a direct activator of the PKC pathway) and ionomycin (a direct regulator of intracellular calcium levels) both at a concentration of 10⁻⁶M, significantly increased Nur77 mRNA gene expression as compared to vehicle-treated controls (figure 4).

Inhibition of the PKA pathway by incubating OC-CM cells with H89 at a concentration of 3×10^{-5} M for 1 hour completely abrogated the increased Nur77 mRNA gene expression observed when OC-CM cell were treated with PGE₂, PGF_{2 α} or FSK (figure 5). When OC-CM cells were incubated overnight with 10⁻⁶M PMA in order to exhaust and therefore inhibit the PKC pathway, Nur77 mRNA gene expression was significantly decreased as compared to OC-CM cells that were incubated overnight with the vehicle only prior to treatment with PGE₂, PGF_{2 α} or PMA (figure 6). Similarly, inhibition of the intracellular calcium pathway by incubation of OC-CM cells with 2×10^{-5} M BAPTA for 1 hour resulted in a significant decrease in Nur77 mRNA gene expression relative to controls as compared to cell cultures pre-incubated with BAPTA vehicle only (figure 7).

DISCUSSION

Exogenous signals alter cell function by inducing changes in gene expression. The first genes to be induced in response to a stimulus are called primary response genes. Primary response genes are expressed rapidly and transiently, and do not require *de novo* protein synthesis (25). We were interested in identifying early changes in the cementoblastic cell gene expression cascade. In order to examine this cascade, OC-CM cells were treated with cycloheximide in order to identify primary response genes. Expression of primary response genes subsequently regulates downstream late gene expression, which may result in phenotypic and functional changes in cells. Therefore, the variations in Nur77 expression when OC-CM cells are treated with prostanoids reported in this study are not due changes in the expression of other genes upstream of Nur77 but due to changes induced by the direct activation of existing cellular proteins in both PKC and PKA signal transduction pathways.

Among several other genes, Nur77 was identified as a primary response gene preferentially induced by flup in cementoblastic cells (22). Nur77 belongs to the family of NGF1-B nuclear orphan receptors, is constitutively expressed in various mammalian tissues(23,26-29) and its expression is induced by treatment of luteal cells with PGF_{2α} (30). The role of Nur77 as transcription factor has been documented on a variety of cell models (23,24,31-34). Hence, based on data obtained from other cell models, Nur77 may potentially play a significant role in cementoblast function. However, no data examining the expression of Nur77 on cementoblasts was available and merited further investigation.

The prostanoids PGE₂, PGF_{2α}, and flup increase, while IL-1 decreases, mineralization by cementoblastic cells (19). The anabolic effect exerted by prostanoids on cementoblastic cells is mediated, as least in part, by induction of the PKC pathway, which occurs through activation of the EP1 and FP prostanoid receptors (19). Induction of the PKA pathway, as observed when cementoblastic cells are treated with parathyroid hormone, decreases mineralization by cementoblastic cells (19). With the Nur77 gene being preferentially induced by flup treatment of cementoblastic cells and not by IL-1, it was hypothesized that its induction played an important role in mineralization by OC-CM cells and that such an induction occurred through activation of the PKC pathway (22).

Herein, a series of experiments was designed to examine the signaling cascades mediating prostanoid-induction of Nur77 in cementoblastic cells. Nur77 mRNA gene expression peaked between 0 and 2 hs following treatment of OC-CM cells with PGE₂ and PGF_{2α} and that expression returned to baseline levels by 4 hs after treatment (figure 1), confirming the primary response characteristics of the gene. Nur77 mRNA gene expression by PGE₂- and PGF_{2α}-treated OC-CM cells was dose-dependent, with maximum effects observed at 10⁻⁵ and 10⁻⁶ M (figure 2), which were concentrations at which these PGs also increased mineralization by OC-CM cells (19). These findings may indirectly provide support to the hypothesis that Nur77 participates in cementoblast function when those cells are exposed to prostanoid agents, as shown previously (19).

Direct induction of the PKC pathway and direct increase in intracellular calcium with PMA and ionomycin, respectively, increased Nur77 mRNA gene expression by OC-CM cells (figures 4 and 6). These agents bypass the prostanoid receptors present on the cell surface and work intracellularly to activate the PKC and calcium signaling pathways. The increase in Nur77 mRNA gene expression by OC-CM cells following treatment with PMA and ionomycin was expected because direct activation of the PKC pathway with PMA treatment of OC-CM cells results in an increase in mineral nodule formation (19) and also because the Nur77 gene is preferentially induced by flup (22), whose effect occurs through activation of the PKC pathway. It is also known that the PKC pathway interacts with the intracellular calcium channel (35).

Release of intracellular calcium stores promotes influx of extracellular calcium via store-operated channels. A rise in cytosolic calcium recruits PKC from the cytosol to the membrane, where diacylglycerol (DAG) activates it. Activation of the PKC depends on both calcium and DAG, suggesting an interaction between the two branches of the inositol-lipid signaling pathway. Therefore, it was not surprising that direct activation of the PKC pathway and an increase in intracellular calcium had similar effects on Nur77 mRNA gene expression by OC-CM cells.

The direct activator of the PKA pathway FSK also significantly increased Nur77 mRNA gene expression by OC-CM cells. There is evidence to support that Nur77 gene induction is regulated through the PKA, PKC and calcium pathways in other organ systems (36-40). Findings from the current study suggest that the mechanism involved in the expression of Nur77 by cementoblastic cells involves similar mechanisms as compared to other types of cells. Direct inhibition of the PKA, PKC and intracellular calcium pathways resulted in abrogation of the induction of Nur77 mRNA gene expression observed when OC-CM cells were treated with FSK, PMA or BAPTA (figures 5, 6 and 7). This further confirms that both intracellular pathways are involved in Nur77 mRNA gene expression by OC-CM cells.

Nur77 mRNA gene expression increased when OC-CM cells were treated with the selective EP1 prostanoid receptor agonist 16,16-DM-PGE₂ and the selective FP prostanoid receptor agonist flup. Both the EP1 prostanoid receptor and the FP prostanoid receptor activate the PKC pathway, which is anabolic in the process of OC-CM mineralization (19). These findings support the theory that the Nur77 gene is induced via activation of the PKC pathway, which occurs through binding of PGs to specific prostanoid receptors on the cell surface. Induction of the Nur77 gene may result in the expression of late response genes, which regulate cell function. Of interest was the fact that sulprostone, a selective agonist of the EP3 prostanoid receptor which signals by inhibiting the PKA pathway, while having an anabolic effect on mineralization by OC-CM cells (19), had no significant effect on the expression of the Nur77 gene in mRNA as compared to non-treated controls. Also, sulprostone-treated OC-CM cells showed significantly less mRNA expression of the Nur77 gene than PGE₂-, PGF_{2α}-, 16, 16-DM-PGE₂ - and flup-treated OC-CM cells. A possible explanation for the fact that activation of the EP3 prostanoid receptor increases mineralization by OC-CM cells but does not increase Nur77 mRNA gene expression is that there may be other primary response genes involved in the cascade of events leading to mineral nodule formation by cementoblastic cells. This proposed mechanism deserves further investigation.

It is also noteworthy that misoprostol, which is an agonist of the EP2, EP3 and EP4 prostanoid receptors did not significantly increase Nur77 gene expression by OC-CM cells. By virtue of binding to EP prostanoid receptors 2 through 4, misoprostol signals in two distinct, yet opposed ways: inducing the PKA pathway (EP2 and EP4 receptors) and inhibiting the PKA pathway (EP3 receptor). Therefore, it is likely that the concomitant activation and inhibition of the PKA pathway neutralized each other, and the net effect on Nur77 gene expression did present with a significant increase.

Butaprost is a selective agonist of the EP2 prostanoid receptor and as such, signals through activation of the PKA pathway. Because direct activation of the PKA pathway with FSK resulted in an increase in Nur77 gene expression by OC-CM cells, treatment of cementoblastic cells with butaprost was also expected to increase expression of the Nur77 gene. The fact that butaprost treatment had no significant effect on Nur77 gene expression by OC-CM cells may be explained by the fact that PKA activation occurs through the EP4 - and not through the EP2 - prostanoid receptor when cementoblastic cells are treated with butaprost. In order to confirm this proposed explanation, Nur77 gene expression by OC-CM cells needed to be examined

under treatment of a selective agonist of the EP4 prostanoid receptor, and such agonist is not currently available.

Taken all together, findings herein support the initial hypothesis that Nur77 mRNA gene expression by cementoblastic cells is modulated by treatment with prostanoid agents and delineate the signaling cascades that mediate such regulation. Combined with published results demonstrating regulation of osteoblastic and cementoblastic differentiation markers by members of the NGIF-B nuclear orphan receptor family (41,42), results from this study suggest that Nur77 gene activation may be involved, at least partially, in OC-CM cell function and differentiation. This involvement possibly occurs by the Nur77 gene regulating late response gene expression, which subsequently causes phenotypic and functional changes on cementoblastic cells. Clarifying the exact mechanism involved in the relationship between PKA activation, Nur77 mRNA gene expression and changes in cementoblastic cell function, warrants further investigation.

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Figure 1.

Nur77 mRNA gene expression by OC-CM cells treated 10^{-6}M PGE₂ and 10^{-6}M PGF_{2α}: time courses. Confluent OC-CM cells were treated with 10^{-6}M PGE₂ or 10^{-6}M PGF_{2α} for 0 to 8 hours. A) PGE₂ treatment: Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. B) PGE₂ treatment: Densitometric values of Nur77 mRNA relative to GAPDH mRNA. C) PGF_{2α} treatment: Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. D) PGF_{2α} treatment: Densitometric values of Nur77 mRNA relative to GAPDH mRNA. Each value is the mean $\pm$ standard error of three experiments. *Significantly different from baseline, $p < 0.05$.

Figure 2.

Nur77 mRNA gene expression by OC-CM cells treated with PGE₂ and PGF_{2α}: dose responses. Confluent cells were treated with various concentrations of PGE₂ and PGF_{2α} for 1 hour. A) PGE₂ treatment: Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. B) PGE₂ treatment: Densitometric values of Nur77 mRNA relative to GAPDH mRNA. C) PGF_{2α} treatment: Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. D) PGF_{2α} treatment: Densitometric values of Nur77 mRNA relative to GAPDH mRNA. Each value is the mean $\pm$ standard error of three experiments. *Significantly different from vehicle-treated control, $p < 0.05$.

Figure 3.

Effect of specific prostanoid receptor activators on Nur77 mRNA gene expression by OC-CM cells. Confluent cells were treated for 1 hour with vehicle (EtOH), 10^{-6} M PGE₂, 10^{-6} M PGF_{2α}, 10^{-7} M butaprost (butap), 10^{-5} M misoprostol (misop), 10^{-6} M fluprostenol, 10^{-6} M 16,16 dimethyl PGE₂ (16,16 PGE₂), 10^{-4} M sulprostone (sulp). A) Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. B) Densitometric values of Nur77 mRNA relative to GAPDH mRNA. Each value is the mean $\pm$ standard error of three experiments. *Significantly different from vehicle-treated control, $p < 0.05$.

Figure 4.

Effect of direct signaling pathway activators on Nur77 mRNA gene expression by OC-CM cells. Confluent cells were treated for 1 hour with vehicle (EtOH), 10⁻⁶M PGE₂, 10⁻⁶M PGF_{2α}, 10⁻⁵M forskolin (FSK – a direct PKA pathway activator), 10⁻⁶M phorbol 12-myristate 13-acetate (PMA – a direct PKC pathway activator), 10⁻⁶M ionomycin (Iono – a regulator of intracellular calcium level). A) Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. B) Densitometric values of Nur77 mRNA relative to GAPDH mRNA. Each value is the mean ± standard error of three experiments. *Significantly different from vehicle-treated control, p < 0.05.

Figure 5. Effect of PKA pathway inhibition on Nur77 mRNA gene expression by OC-CM cells. Confluent OC-CM cells were incubated for 1 hour with vehicle or 3×10^{-5} M H89 (a PKA signaling pathway inhibitor) and then treated with 10^{-6} M PGE₂, 10^{-6} M PGF_{2α} or 10^{-5} M forskolin (FSK – a direct PKA signaling pathway activator) for 1 hour. A) Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. B) Densitometric values of Nur77 mRNA relative to GAPDH mRNA. Each value is the mean $\pm$ standard error of three experiments. *Non-H89 treated group: significantly different from vehicle-treated control, $p < 0.05$. #H89 vs. non-H89 treated groups: significantly different, $p < 0.05$.

Figure 6.

Effect of PKC pathway inhibition on Nur77 mRNA gene expression by OC-CM cells. Confluent OC-CM cells were incubated overnight with vehicle or 10⁻⁶M phorbol 12-myristate 13-acetate (PMA – a direct PKC pathway activator) as means to exhaust the PKC pathway and then treated with 10⁻⁶M PGE₂, 10⁻⁶M PGF_{2α} or 10⁻⁶M PMA for 1 hour. (A) Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. (B) Densitometric values of Nur77 mRNA relative to GAPDH mRNA. Each value is the mean $\pm$ standard error of three experiments. *Non-PMA treated group; significantly different from vehicle-treated control, $p < 0.05$. #PMA vs. non-PMA treated groups; significantly different $p < 0.05$.

Figure 7.

Effect of intracellular calcium pathway inhibition on Nur77 mRNA gene expression by OC-CM cells. Confluent OC-CM cells were incubated for 1 hour with vehicle or 2×10^{-5} M 1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA – a direct intracellular calcium pathway inhibitor) and then treated with 10^{-6} M PGE₂, 10^{-6} M PGF_{2α} or 10^{-6} M PMA for 1 hour. (A) Northern blot analysis of Nur77 and GAPDH mRNA levels and ethidium bromide staining of RNA. (B) Densitometric values of Nur77 mRNA relative to GAPDH mRNA. Each value is the mean $\pm$ standard error of three experiments. *Non-BAPTA treated group: significantly different from vehicle-treated control, $p < 0.05$. #BAPTA vs. non-BAPTA treated groups; significantly different $p < 0.05$.