Transcriptional Regulation of Human Prostaglandin-endoperoxide Synthase-2 Gene by Lipopolysaccharide and Phorbol Ester in Vascular Endothelial Cells

INVolvement of both nuclear factor for interleukin-6 expression site and cAMP response element*

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There exist two distinct isozymes of prostaglandin-endoperoxide synthase (PES). PES-2 mRNA is synergistically induced by lipopolysaccharide (LPS) and 12-O-tetradecanoylphorbol-13-acetate (TPA) in bovine arterial endothelial cells. On the other hand, PES-1 mRNA is constitutively expressed under these conditions. Therefore, the promoter activities of the human genes for PES-1 and -2 in bovine arterial endothelial cells were examined. The 5′-flanking region of the human PES-2 gene (nucleotides −327 to +59) showed promoter activity inducible by LPS and TPA using transient transfection analysis, whereas that of the PES-1 gene (nucleotides −1010 to +69) showed constitutive promoter activity. Destruction of both consensus sequences for the nuclear factor responsible for the interleukin-6 expression (NF-IL6) site (nucleotides −132 to −124) and the cyclic AMP response element (CRE) (nucleotides −59 to −53) of the human PES-2 gene markedly reduced the promoter activity (25%) of the PES-2 gene after combined treatment with LPS and TPA, although single destruction of the NF-IL6 site or the CRE slightly reduced the promoter activity (60% or 90%, respectively). Moreover, cotransfection experiments showed that a trans-acting factor, CCAAT enhancer binding protein (C/EBPδ), which binds to both the NF-IL6 site and the CRE, increased the promoter activity of the PES-2 gene mainly through the CRE. C/EBPδ mRNA was rapidly induced by LPS. Collectively, these results suggest that transcription of the PES-2 gene in vascular endothelial cells is regulated through combination of the NF-IL6 site and the CRE and that C/EBPδ functions as one of the trans-acting factors.

Prostaglandin-endoperoxide synthase (PES) catalyzes the first step of the biosynthesis of prostanoids such as prostaglandins, thromboxane and prostacyclin, and is an effective pharmacological target for nonsteroidal antiinflammatory drugs such as aspirin. There exist two distinct isozymes for PES, PES-1 and PES-2. The human PES-1 gene, mapped to chromosome 9q32-q33.3, is about 22 kilobase pairs (kb) in size with 11 exons (4, 5), whereas the human PES-2 gene, mapped to chromosome 1q25.2-q25.3, is about 8.3 kb in size with 10 exons (5). PES-2 mRNA is induced by a variety of factors, i.e. inflammatory mediators, growth factors, mitogens, and hormones (6–13), whereas expression of PES-1 mRNA is generally constitutive (14). From these findings, it seems likely that PES-2 and PES-1 play distinct roles in the production of prostanoids.

Studies on the transcriptional regulation of eukaryotic genes have led to identification of a number of transcription factors that are mediated through specific cis-acting elements. Transcriptional activation in response to extracellular signals involves the regulated assembly of multiprotein complexes on enhancers and promoters (15). In the human PES-2 gene, the nucleotide sequence of the 5′-flanking region with a canonical TATA box (5) does not show similarity to that in the human PES-1 gene with no TATA box (16), which likely reflects the distinct expression patterns of the two genes. Consensus sequences of the nuclear factorκB (NF-κB) site, the nuclear factor for interleukin-6 expression (NF-IL6) site and the cyclic AMP response element (CRE) are found in the 275-bp region upstream from the transcriptional start site in the human PES-2 gene (5). The sequences homologous to the consensus NF-IL6 site and CRE are also found in the corresponding regions of the mouse (17) and rat (13) PES-2 genes. Trans-acting factors binding to the NF-IL6 site have several isoforms with a leucine zipper motif for dimer formation (18–21), i.e. C/EBPα, C/EBPβ (NF-IL6), and C/EBPδ (NF-IL6δ). Siros and Richards (22) have reported that C/EBPδ may play a key role in regulating induction of the PES-2 gene in rat granulosa cells (22). On the other hand, we have reported that the CRE is essential for expression of the human PES-2 gene in monocytic-differentiated U937 cells (23). Xie et al. have also reported that v-src induction of the PES-2 gene is mediated by the CRE in NIH 3T3 cells (24). Several factors that specifically recognize the CRE have also been identified as trans-acting factors with a leucine zipper motif for dimer formation (25). Interestingly, rat C/EBPδ cDNA has been isolated as a trans-acting factor that binds to the CRE of the substance P precur-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D64068.

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‡ The abbreviations used are: PES, prostaglandin-endoperoxide synthase; CRE, cAMP response element; NF-IL6, nuclear factor for interleukin-6 expression; NF-κB, nuclear factorκB; bp, base pairs(s); kb, kilobase pair(s); BAEC, bovine arterial endothelial cell(s); DMEM, Dulbecco’s modified Eagle’s medium; LPS, lipopolysaccharide; TPA, 12-O-tetradecanoylphorbol-13-acetate; DMEM-SF, serum-free and antibiotic-free DMEM; C/EBP, CCAAT enhancer binding protein.
sor gene by expression cloning (26).
Moreover, heterodimer formation of NF-IL6 (C/EBPβ) with CRE-binding protein has been suggested in the composite NF-IL6-CRE binding sites of the human prointerleukin 1β gene (27). These reports have raised the possibility of complex formation among distinct trans-acting factors binding to several cis-acting elements in the PES-2 gene.

In the present study, we first investigated the promoter activities of both the human PES-2 and PES-1 genes in bovine arterial endothelial cells (BAEC) using a transient transfection method and found that the expression patterns of these genes induced by LPS and TPA were primarily ascribable to the transcriptional activities of their 5'-flanking regions. Secondly, we found that the NF-IL6 site and the CRE (nucleotides -132 to -124 and -59 to -53) are involved cooperatively in the promoter activity of the PES-2 gene and that C/EBPα, which binds to both the NF-IL6 site and the CRE, is suggested to function as a trans-acting factor in the human PES-2 gene.

MATERIALS AND METHODS

Cell Culture—BAEC were obtained from Dr. Michitaka Masuda (National Cardiovascular Center, Osaka). BAEC were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, Scotland), 50 μU/ml penicillin, and 100 μg/ml streptomycin sulfate in a humidified atmosphere of 5% CO2 in air. TPA and LPS (from Escherichia coli serotype 055, B5) were obtained from Sigma and used at concentrations of 100 μM and 10 μg/ml in the medium, respectively.

Plasmid Construction—pGV-B, the promoter-less luciferase reporter vector, and pGV-C, the luciferase reporter vector under control of the SV40 promoter/enhancer, were purchased from Toyko Ink, Tokyo. These expression vectors were used as controls in the transfection experiments. The recombinant phagemid phPES20 (4) containing the human PES-1 genomic sequence was characterized by restriction mapping, and suitable restriction fragments were subcloned into the pBluescript II SK+, a plasmid known for its ability to generate restriction map sizes. The result showed that the 1079-bp fragment of the genomic DNA insert from phPES20 digested with SacI contained the 5'-flanking region of the human PES-1 gene and covered the nucleotide positions -1010 to +69 from the reported transcription start site (16) as +1. This DNA fragment was ligated into the SacI site of pG-B DNA with a sense orientation, and this construct was designated phPES1 (1010 to +69). Constructions of reporter vectors for the human PES-2 gene, such as phPES2(-1432 to +59), phPES2(-327 to +59), phPES2(-220 to +59), phPES2(-124 to +59), and phPES2(-52 to +59), were described previously (23). Mutant constructs, phPES2(CRM) with a mutation at the CRE, phPES2(CRM) with a mutation at the CRE, and phPES2(ILM), with a mutation at the NF-IL6 site, were constructed by site-directed mutagenesis using the QuickChange Site-Directed Mutagenesis Kit (Stratagene). The oligonucleotides PES2CRM and PES2NFILM were prepared by the same method as phPES2(CRM) using phPES2(CRM) as a polymerase chain reaction template instead of phPES2(-327 to +59). The oligonucleotide primers were synthesized on an Applied Biosystems 391 DNA synthesizer. Each construct was verified by DNA sequence analysis as described above (5). Plasmids expressing C/EBPβ family members (20) or C/EBPβ, C/EBPβ, and C/EBPβ, under control of the mouse sarcoma virus promoter were kindly provided by Dr. Steven L. McKnight (Tulane Inc.).

RNA Blot Analysis—RNA blot analysis was performed as described previously (5). Total RNA was isolated according to the acid guanidinium thiocyanate procedure (28), and poly(A)+ RNA was purified by oligo(dT)30-latex (Nippon Roche). 24 μg of total RNA of 104 cells/well were loaded onto a 1% agarose gel containing 2.2 M formaldehyde and 10 μg/ml of RNA size marker. The RNA was denatured, transferred to a positively charged nylon membrane, and hybridized with 32P-labeled probes. The DNA blots were stripped of radioactivity by incubation with 0.1% SDS at 96°C for 3 min. Bovine mRNA for PES-1 or PES-2 was detected specifically as a single band (3 kb for PES-1 or 4 kb for PES-2) using these probes, respectively. The mRNA levels were calculated on the basis of hybridization signals measured by a Fujix BAS2000 imaging analyzer (Fuji Photo Film Co., Tokyo).

DNA Transfections—Triplicate BAEC cultures (8 × 105 cells/well) on 12-well tissue culture plates (Corning) were cultivated 1 day before transfection. In order to measure transfection efficiency, cells were cotransfected with pCMV-β-gal and a β-galactosidase activity under control of the human cytomegalovirus promoter (23). For each well, 0.4 μg of the reporter vector of interest and 0.2 μg of pCMV-β-gal were added to 40 μl of serum-free and antibiotic-free DMEM (DMEM-SF), mixed with 4.8 μl of Lipofectin (Life Technologies, Inc.) in 40 μl of DMEM-SF, and incubated at 22°C for 15 min, and then 0.32 ml of DMEM-SF was added. BAEC were washed with 1 ml of DMEM-SF, 0.4 ml of the DNA-lipofectin complex was added, and the cells were incubated for 5 h at 37°C. At this time, the medium was changed to complete medium (20% fetal calf serum), and the cells were cultivated for 43 h with another change of the medium (10% fetal calf serum) on the following day. 48 h after transfection, the cells were treated by a change of medium containing no mediator (control), TPA, LPS, or a combination of both for 5 h. The cells were harvested, and their luciferase and β-galactosidase activities were determined by a luminometer (Berthold) (29) and a method using chlorophenol red β-d-galactopyranoside as a substrate (30). Cotransfection experiments using an effector plasmid expressing C/EBPα, β, or γ with a luciferase reporter plasmid were performed in the same way as above and harvested 48 h after transfection. In these experiments, the amount of effector DNA made constant (0.6 μg/well) by adding pGV-B DNA if necessary. For the gel-shift assay, transfection was scaled up using 35-mm dishes instead of 12-well tissue culture plates (3 μg/dish of an effector plasmid expressing C/EBPα, β, or γ, 0.5 μg/dish of pCMV-β-gal, and 27 μl/dish of lipofectin).

Nuclear Extracts and Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared as described by Andrews and Falleri (31). The following oligonucleotides were used as a DNA synthesizer: 5'-AAAGCAGCTATTCGGTCATACGGGTTG-3', containing the consensus CRE sequence of the human PES-2 gene indicated by underlining (PES2CRE); 5'-AAACAGTCTTGGAGCCATACGGGTTG-3', containing a four-point mutation (indicated by lower case letters) within the consensus CRE sequence by introducing a SacI site (underlined) (PES2CREmut); 5'-GGGCTTACGCAATTTTTTTAA-3', containing the consensus NF-IL6 site (indicated by underlining (PES2NFIL6); 5'-ACGGGTACCGAATTTTTTTAA-3', containing a five-point mutation (indicated by lower case letters) within the consensus NF-IL6 site by introducing a KpnI site (PES2NFIL6). The oligonucleotides PES2CRM and PES2NFIL6 have sequences identical to the reporter vectors phPES2(CRM) and phPES2(ILM), respectively. Complementary oligonucleotides synthesized separately were annealed in 20 mM Tris-HCl (pH 7.6), 50 mM NaCl, 10 mM MgCl2, and 1 mM dithiothreitol. The annealed oligonucleotides were phosphorylated at the 5'-end with [γ-32P]ATP (110 TBq/mmol, Amershams) and T4 polynucleotide kinase. Electrophoretic mobility shift assays using synthesized oligonucleotides were carried out as described previously (29).

RESULTS

Effects of TPA and LPS on Expression of PES-2 and PES-1 mRNA in BAEC—It has been reported that stimulation of human umbilical vein endothelial cells with TPA or LPS increased the levels of mRNA for PES-2 and that this change correlated well with increased prostacyclin biosynthesis (9). We first investigated whether PES-2 and -1 were induced in BAEC following stimulation with TPA, LPS, or a combination of both. As shown in Fig. 1A, PES-2 mRNA was detected as a faint band in control cells, and a 5-, 6-, or 14-fold increase in PES-2 mRNA relative to control cells was observed after a 5-h stimulation with TPA, LPS, or a combination of both, respectively. In contrast with PES-2, PES-1 mRNA was detected as a clear band in control cells, and the expression level did not show a significant increase after 5 h of stimulation (Fig. 1B). These results showed that expression of PES-2 mRNA was synergistically induced by LPS and TPA, whereas expression of PES-1 mRNA was not changed with these treatments in BAEC.

Functional Promoter Activity of the Human PES-2 or PES-1 Gene in BAEC—To determine if the 5'-flanking regions of the human PES-2 and -1 genes contain functional domains for...
their promoters, transient DNA transfection experiments using luciferase as a reporter gene in BAEC were performed (Fig. 2). As shown in Fig. 2A, the promoter activity observed with the reporter vector phPES2(−327/+59), which contains the 5′-flanking region of the human PES-2 gene, was about 200% of that with the pGV-C expression vector containing the SV40 promoter/enhancer. This promoter activity with phPES2(−327/+59) was induced 1.7-, 2.8-, and 6.4-fold relative to control cells by TPA, LPS, or a combination of both, respectively. The promoter activity with phPES2(−1432/+59) was about 75% of that with phPES2(−327/+59) and was induced 1.8-, 3.2-, or 7.3-fold relative to control cells by TPA, LPS, or a combination of both, respectively. These results showed that nucleotides −327/+59 of the human PES-2 gene (Fig. 3A) functioned as the inducible promoter for both TPA and LPS, accounting primarily for the inducible expression of PES-2 mRNA. On the other hand, as shown in Fig. 2B, the luciferase activity observed with the reporter vector phPES2(−1010/+69), which contains the 5′-flanking region of the human PES-1 gene, was about 8% of that with the pGV-C expression vector. However, it was significantly higher than that of the promoterless expression vector pGV-B and was not changed by stimulation with TPA, LPS, or a combination of both. From these results, nucleotides −1010 to +69 of the human PES-1 gene (Fig. 3B) appear to function as the constitutive promoter accounting for constitutive expression of PES-1 mRNA.

Analysis of the Region Responsible for the Promoter Activity of the PES-2 Gene—To determine which region is functional for the promoter activity of the human PES-2 gene, transient DNA transfection experiments with BAEC were performed using deletion and site-specific mutants of the human PES-2 promoter (Fig. 4). The promoter activity obtained with phPES2(−52/+59) was about 1% of that with phPES2(−327/+59), and almost no induction was observed upon stimulation with TPA or LPS. The promoter activity with phPES2(−124/+59) or phPES2(−220/+59) was about 13 or 65% of that with phPES2(−327/+59), respectively. The induction pattern roughly similar to that for phPES2(−327/+59) was observed upon stimulation with TPA, LPS, or a combination of both. These results indicated that the region −53 to +59 has basal promoter activity and that at least three regions containing the CRE, NF-IL6 site, and NF-kB site, respectively, are involved in the inducible promoter activity. From previous reports (22–24), the CRE and NF-IL6 site are likely to be involved in the promoter activity. To examine this possibility upon stimulation with TPA and LPS in BAEC, a reporter vector phPES2(CRM) with a mutation at the CRE, or phPES2(ILM) with a mutation at the NF-IL6 site, was transfected into BAEC. The promoter activity obtained with phPES2(CRM) or phPES2(ILM) was about 58% of that with the wild-type phPES2(−327/+59). The induction pattern roughly identical to that for the wild-type was observed, indicating that single destruction of the CRE or NF-IL6 site did not affect the promoter activity significantly. However, the induced promoter activity of phPES2(CRM) or phPES2(ILM) by combination of TPA and LPS was about 90 or 60% of that with phPES2(−327/+59), respectively, suggesting a functional difference in promoter activity between the CRE and NF-IL6 site. Next, a reporter vector phPES2(CRM,ILM) with mutations at both the CRE and NF-IL6 site was transfected into BAEC. The promoter activity obtained with phPES2(CRM,ILM) was decreased to about 25% of that with the wild-type, but it still showed a similar induction pattern. These results showed that the CRE and NF-IL6 site cooperatively affected the promoter activity, although other site(s) might also be involved.

Activation of the PES-2 Promoter by C/EBPδ through both the CRE and NF-IL6 Site—Trans-acting factors binding to the NF-IL6 site have several isoforms, i.e., C/EBPα, C/EBPβ, and C/EBPδ (18–21). The heterodimer formation of NF-IL6 (C/EBPδ) with CRE-binding protein has been reported in the composite NF-IL6-CRE binding site of the human prointerleukin 18 gene (27). Therefore C/EBP family members will possibly bind to both the NF-IL6 site and the CRE in the human PES-2 gene and contribute to its regulation. To test this possibility, electrophoretic mobility shift assays were performed using the oligonucleotides corresponding to the CRE (PES2CRE) and the NF-IL6 site (PES2NIFIL6) and their mutants (PES2CRM and PES2NIFILM) (Fig. 5A). When nuclear extract from the control BAEC was used with 32P-labeled PES2CRE as a probe, two faint but distinct bands were mainly observed (Fig. 5A).
Results indicated that C/EBPα interacts, but not by their mutants, respectively (Fig. 5). Lysed, and assayed for both luciferase and β-galactosidase activities. However, when nuclear extract from BAEC cotransfected with each reporter plasmid together with pCMV-α and this increase was dependent on the amount of the effector C/EBPα, indicating that C/EBPα increased the promoter activity mainly through the CRE (nucleotides −59 to −53).

Fig. 3. Schematic representation of the 5′-flanking region of the human PES-2 (A) or PES-1 (B) gene. Potential cis-acting elements in nucleotides −327/+59 of the human PES-2 gene (A) and nucleotides −1010/+59 of the human PES-1 gene (B). The sequences of up to −1681 bp of the human PES-2 gene (S) and up to −896 bp of the human PES-1 gene (P) reported previously. We determined nucleotide sequence −1010/+59 of the human PES-1 gene in this study. The diagrams show the potential response elements based on sequence similarities to consensus response elements. Distances are given as nucleotide positions relative to the transcriptional start site as +1.

Involvement of CEBPα in Induction of PES-2 mRNA by LPS—Next we investigated whether CEBPα was involved in the expression of the human PES-2 gene induced by TPA, LPS, or a combination of both. As shown in Fig. 8A, CEBPα mRNA was detected as a faint band (1.1 kb) in control cells, and a 1.2- to 8.6- or 4.2-fold increase in CEBPα mRNA relative to control cells was observed after a 4-h stimulation with TPA, LPS, or a combination of both, respectively. In addition, another band (2.1 or 4.1 kb) was detected after stimulation with LPS or a combination of both. In contrast, CEBPα mRNA was constantly expressed as a single band (about 1.8 kb) by these treatments (Fig. 8B). Moreover, as shown in Fig. 9A, induction of PES-2 mRNA by LPS in BAEC was not transient but sustained for at least 24 h, which was distinct from the transient induction by TPA in Swiss 3T3 cells (6). Furthermore, as shown in Fig. 9B, cycloheximide did not potentiate the induction of PES-2 mRNA by LPS or TPA/LPS, although cycloheximide alone induced PES-2 mRNA. Taken together, it is suggested that induced CEBPα plays a role, at least, for sustained induction of PES2 mRNA by treatment with LPS.
DISCUSSION

The promoter activity obtained with the human PES-1 gene using the nucleotides −1010 to −69 in BAEC was 8% of that of SV40 promoter/enhancer, and this activity was similar to that reported previously using nucleotides −898 to +12 of the human PES-1 gene in murine neuroblastoma NS-20 cells (5% of the activity of the SV40 early promoter) (16). On the other hand, the promoter activity of the human PES-2 gene obtained using nucleotides −1432 to +59 was 15-fold higher than that of the PES-1 gene in BAEC (Fig. 2). This result was different from the finding (Fig. 1) that the amount of PES-1 mRNA was greater than that of PES-2 mRNA in the control BAEC. This difference may be due to instability of the PES-2 mRNA conferred by 17 copies of Shaw-Kamen sequence (AUUUA) in the 3'-untranslated region (5, 6, 13), which is found in many immediate-early genes and have been shown to enhance mRNA degradation (32). Ristimäki et al. (33) have reported that post-transcriptional mechanisms are also important in the sustained induction of PES-2 mRNA (33). However, the possibility remains that transcriptional inhibition of the PES-2 gene occurs in regions other than nucleotides −1432 to +59.

Recently, it was suggested that transcriptional regulation may be conducted through the cooperation of more than one trans-acting factor with regulated assembly of multiprotein complexes on enhancers and promoters. The complex nature of these processes is considered to result in an elaborate fail-safe...
mechanism for controlling gene expression (15). In this study, we showed that combination of the CRE and the NF-IL6 site in the human PES-2 gene was involved in promoter activity, although single destruction of the CRE or the NF-IL6 site reduced the activity slightly (Fig. 4). A similar result has also been obtained in transfection experiments using human umbilical vein endothelial cells (data not shown). On the other hand, destruction of both the CRE and the NF-IL6 site could completely eliminate the inducible promoter activity (Fig. 4), suggesting the need to examine the contribution of other cis-acting element(s), although we could not exclude from the possibility that the CRE and the NF-IL6 site modulate the basal promoter activity not the inducible promoter activity. These results can be explained by the fail-safe mechanism of gene expression resulting from complex formation between transcription factors through the CRE, the NF-IL6 site, and other cis-acting element(s). In fact, the promoter activity with phPES2(CRM) was increased as little as 2-fold by expression of C/EBPδ, although with the wild-type phPES2(−327/+59) was increased about 7-fold as shown in Fig. 7. From these results, we interpreted that C/EBPδ was a component of transcription factor, which increased the PES-2 promoter activity mainly through the CRE, but in the induction by LPS or TPA/LPS, the increased promoter activity by C/EBPδ through the CRE was likely to be complemented by other transcription factor(s) through NF-IL6 site or other cis-acting element(s).

The NF-IL6 site may be a candidate of such cis-acting elements, since NF-IL6 (C/EBPβ) cDNA has been isolated as a transacting factor that binds to the p50 subunit of NF-κB (34). C/EBPδ, a DNA-binding protein with affinity for both the CRE and the NF-IL6 site (Fig. 5), was demonstrated to activate the PES-2 promoter mainly through the CRE (Fig. 7). CRE-binding proteins have been reported to bind to two classes of CRE: (i) symmetrical CRE (5′-TGACGTCA-3′), consisting of two overlapping GTCTA palindromic half-sites such as those found in the somatostatin (35) and α-chlorionic gonadotropin (36) genes, and (ii) asymmetrical CRE, consisting of the sequence GTCTA, representing a single perfect half-site such as that present in the human PES-2 gene, as shown in Fig. 5A. Very recently, the asymmetrical CRE recognized by the heterodimer of NF-IL6 (C/EBPβ) with CRE-binding protein was found in the human prointerleukin 1β gene as the LPS-responsive enhancer (27). Electrophoretic mobility shift assays showed that the C/EBPδ expressed in BAEC recognized both PES2CRE and PES2NFIL6 (Fig. 5, A and C) but did not recognize the symmetrical CRE of somatostatin (data not shown), suggesting the possibility of heterodimer formation of C/EBPδ with CRE-binding protein on the CRE of the human PES-2 gene. On the other hand, expression of C/EBPδ mRNA was rapidly induced by LPS but not by TPA (Fig. 8). As shown in Fig. 1, LPS and TPA synergistically induced PES-2 mRNA in BAEC. In fact, the amount of PES-2 mRNA was increased by LPS in a concentration-dependent manner up to 10 μg/ml, but
it was less than 50% of that induced with a combination of 10 μg/ml LPS and 100 nm TPA (data not shown). Interestingly, induction of PES-2 mRNA by LPS in BAEC was not transient but sustained for at least 24 h, which essentially agreed with the induction pattern by interleukin-1α in human umbilical vein endothelial cells (33), but was distinct from the transient induction by TPA in Swiss 3T3 cells (6). Furthermore, as shown in Fig. 9B, cycloheximide did not potentiate the induction of PES-2 mRNA by LPS, although cycloheximide alone induced PES-2 mRNA. Taken together, it is suggested that C/EBPβ plays a role, at least, in sustained induction of PES2 mRNA by treatment with LPS. It is also possible that C/EBPβ participates in the initial step of induction of PES-2 mRNA since phosphorylation of C/EBP family members was reported to participate in transcriptional regulation of several genes (27). However, further study is necessary for understanding the function of C/EBPβ in the expression in vivo of PES-2 gene.

It has been reported that C/EBPβ may play a key role in regulating the induction of the PES-2 gene in rat granulosa cells prior to ovulation (22). This contradicts our finding that coexpression of C/EBPβ decreased the promoter activity of the PES-2 gene in BAEC (Fig. 6). On the other hand, we previously reported that the promoter activity obtained with pPES2(CRM) or pPES2(ILLM) was about 20 or 80% of that with pPES2(−327/+59) in differentiated U937 monocyctic cells, respectively (23). This was also different from our finding that the promoter activity obtained with pPES2(CRM) or pPES2(ILLM) was about 58% of that with pPES2(−327/+59) in BAEC (Fig. 4). These differences may reflect distinct complex formation by trans-acting factors in distinct cells (BAEC, U937 cells, and granulosa cells).

In summary, we have first shown that the expression patterns of the human PES-2 and PES-1 genes are primarily accounted for by the transcriptional activities of their 5′-flanking regions. Second, the NF-IL6 site and the CRE were shown to be cooperatively involved in the promoter activity of the PES-2 gene inducible by LPS and TPA, although other cis-acting element(s) will also be necessary to explain the inducible promoter activity. Third, we have suggested that C/EBPβ, as a trans-acting factor, is involved in activation of the PES-2 promoter through the CRE by LPS.

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