Identification of a Prostaglandin-responsive Element in the Na,K-ATPase β₁ Promoter That Is Regulated by cAMP and Ca²⁺: Evidence for an Interactive Role of cAMP Regulatory Element-Binding Protein and Sp1

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The Na,K-ATPase is a transmembrane protein responsible for maintaining electrochemical gradients across the plasma membrane in all mammalian cells, a process that is subject to regulation at the transcriptional as well as post-transcriptional level. Included among physiologic regulators in the kidney are prostaglandins. Previously, we demonstrated that prostaglandin E₁ (PGE₁) increases the activity and expression of the Na,K-ATPase in Madin-Darby canine kidney cells (Taub, M., Borsick, M., Geisel, J., Matlhagela, K., Rajkhowa, T., and Allen, C. (2004) Exp. Cell Res. 299, 1–14; Taub, M. L., Wang, Y., Yang, I. S., Fiorella, P., and Lee, S. M. (1992) J. Cell. Physiol. 151, 337–346). In this work, we present evidence that transcription of the Na,K-ATPase β₁ subunit is stimulated by PGE₁, an effect that may be mediated through the cAMP and Ca²⁺ pathways. Transient transfection studies using 5'-deletion mutants of the human β₁ subunit promoter indicated that region −100 to −92 containing the sequence GAGCCCTGC (a prostaglandin-responsive element (PGRE)) is required to elicit the stimulatory effects of PGE₁. 8-bromo-cAMP, phorbol 12-myristate 13-acetate, and okadaic acid. Electrophoretic mobility shift assays indicated that both the cAMP regulatory element-binding protein (CREB) and Sp₁ bind to the PGRE present within this region of the β₁ subunit promoter. The involvement of the PGRE and Sp₁ sites in regulation by PGE₁ was further confirmed by the increased PGE₁ stimulation that was observed following insertion of the PGRE into a promoter/luciferase construct containing a portion of a heterologous promoter and the fibronectin promoter with four GC boxes. Further evidence suggesting an interaction between Sp₁ and CREB was obtained from experiments conducted with pLuc-MCS-β72–167, which contains region −167 to −72 in the human β₁ subunit promoter. The PGE₁ stimulation observed in Madin-Darby canine kidney cells transiently transfected with pLuc-MCS-β72–167 was reduced when the two GC boxes immediately upstream from the PGRE were translocated farther upstream. Also consistent with an interaction between CREB and Sp₁ are the results of our immunoprecipitation studies indicating that CREB co-immunoprecipitated with Sp₁ when an antibody against CREB, Sp₁, or the CREB-binding protein was used.

The Na,K-ATPase is a ubiquitous transmembrane protein that establishes and maintains an electrochemical gradient across the plasma membrane in virtually all mammalian cells (3). In addition, the Na,K-ATPase plays specialized roles in specific cell types. For example, in nerve and muscle cells, the Na,K-ATPase is involved in the propagation of an action potential. In the kidney, where the Na,K-ATPase is present at higher levels than in most tissues, the Na,K-ATPase plays an important role in Na⁺ reabsorption, a process that occurs in tubular epithelial cells. In kidney tubule epithelial cells, the Na,K-ATPase is present in the basolateral membrane, promoting transepithelial ion transport (4). Na⁺ in the lumen of the nephron is transported into the tubule epithelial cells through transport systems localized to the apical membrane, including the Na⁺/H⁺ antiport system. Intracellular Na⁺ is then transported out of the cells’ basolateral membrane through the Na,K-ATPase in exchange for extracellular K⁺, a process that utilizes ATP. The α subunit of the Na,K-ATPase (a 112-kDa protein) mediates the catalytic activity, whereas the glycosylated β subunit (40–60 kDa) has been proposed to facilitate the assembly and transport of the α subunit from the endoplasmic reticulum to the plasma membrane.

The Na,K-ATPase is subject to regulation by a number of effector molecules, including peptide hormones such as triiodothyronine (T₃)¹ and steroid hormones such as hydrocortisone. These effector molecules may have acute effects on the activity of pre-existing Na,K-ATPases (5) as well as chronic effects resulting from changes in the number of Na,K-ATPases present intracellularly (2). Such chronic effects may be the consequence of transcriptional and/or post-transcriptional events. When considering the effects of any one of a number of regulatory molecules, their effects on the Na,K-ATPase are difficult to predict, as exemplified by the case of T₃. In fetal rat brain, T₃ affects gene expression largely at the post-transcriptional level (6). However, in rat kidney cortex, T₃ also affects Na,K-ATPase α₁ and β₁ subunit gene expression at the translational level (7). The mechanisms by which other regulatory factors control Na,K-ATPase activity are often not similarly well defined.

Prostaglandins are included among the regulatory factors that regulate the activity of the Na,K-ATPase (5, 8–10). Prosta-

¹ The abbreviations used are: T₃, triiodothyronine; PKC, protein kinase C; PGE₁, prostaglandin E₁; MDCK, Madin-Darby canine kidney; PGRE, prostaglandin response element; SPGRE, Sp₁-containing PGRE; 8-Br-cAMP, 8-bromo-cAMP; PMA, phorbol 12-myristate 13-acetate; CRE, cAMP-responsive element; CREM, CRE modulator; CREB, cAMP regulatory element-binding protein; CBP, CREB-binding protein; GST, glutathione S-transferase; DME/M, Dulbecco’s modified Eagle’s medium; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine; FN, fibronectin; MRE/CRE, mineralocorticoid/glucocorticoid-responsive element; TRE, thyroid hormone-responsive element; CaMK, Ca²⁺/calmodulin-dependent protein kinase; ATM, activating transcription factor.
taglndinites initiate their regulatory effects on transport systems such as the Na,K-ATPase through their interaction with G-protein-coupled prostanoid receptors. Subsequently, such signaling pathways as the cAMP and protein kinase C (PKC) pathways may be activated depending upon the prostanoid receptor that is involved.

This study is concerned with defining the transcriptional regulation of the Na,K-ATPase by one such prostaglandin, PGE1, using a well characterized in vitro model system of the renal distal tube, the Madin-Darby canine kidney (MDCK) cell line (11). Previously, we observed that PGE1 stimulates the activity of the Na,K-ATPase in MDCK cells, an effect that could be explained by increased expression of the mRNAs for the α and β subunits of the Na,K-ATPase (1, 2). We have also obtained evidence of a stimulatory effect of PGE1 on β1 subunit transcription while using a human Na,K-ATPase β1 promoter/luciferase construct (pHj1–1141Luc (12)).

In this work, we define a prostanoid-responsible element (PGRE) that is present in the human Na,K-ATPase β1 subunit promoter. The results of our transient transfection studies with 5′-deletion mutants of the β1 subunit promoter indicate the presence of a functional PGRE with the sequence AGTC-CCTGC at a position located within region −182 to −83. The observed 8-bromo-cAMP (8-Br-cAMP) stimulation, the phorbol 12-myristate 13-acetate (PMA) stimulation, and the okadaic acid stimulation, like the PGE1 stimulation, were all dependent upon the presence of the same functional element in the β1 subunit promoter, suggesting that this element is a cAMP-responsive element (CRE). Electrophoretic mobility shift assays indicated the presence of a sequence located between −111 and −81 that binds to the cAMP regulatory element-binding protein (CREB) and Sp1 transcription factors. To determine further the nature of the involvement of CREB and Sp1 in mediating PGE1 stimulation, the PGRE was introduced into a heterologous promoter, and studies were conducted with pLuc-MCS containing either a normal or a mutant region of the β1 subunit promoter. In addition, evidence for an interaction between CREB and Sp1 was obtained from immunoprecipitation and glutathione S-transferase (GST) pull-down studies. The results of these studies suggest that a novel interaction between these transcription factors is involved in mediating regulation initiated by effector molecules acting through both cAMP- and Ca2+-mediated pathways.

**EXPERIMENTAL PROCEDURES**

**Materials**—Hormones, human transferrin, PGE1, and other chemicals were from Sigma. Custom-designed double-stranded synthetic oligonucleotides (PAGE-purified), Dulbecco’s modified Eagle’s medium (DMEM), nutrient mixture F-12, soybean trypsin inhibitor, the RNA galactonoligosaccharides (PAGE-purified), Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen), [32P]dATP and BioMax MS-2 film was from Amersham Biosciences. The pcDNA3-Sp1 expression vector containing the sequence corresponding to amino acids 121–345 mapping near the N terminus of Sp1 in mediating PGE1 stimulation, the PGRE was introduced into a heterologous promoter, and studies were conducted with pLuc-MCS containing either a normal or a mutant region of the β1 subunit promoter. In addition, evidence for an interaction between CREB and Sp1 was obtained from immunoprecipitation and glutathione S-transferase (GST) pull-down studies. The results of these studies suggest that a novel interaction between these transcription factors is involved in mediating regulation initiated by effector molecules acting through both cAMP- and Ca2+-mediated pathways.

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**Cell Culture and Transfection**—The basal medium consists of a 50:50 mixture of DMEM and Ham’s nutrient mixture F-12 supplemented with 15 mM HepES (pH 7.4), 20 mM sodium bicarbonate, penicillin, and streptomycin as described previously (17). The growth medium for stock cultures of MDCK cells (Medium K-1) was further supplemented with 5 μg/ml bovine insulin, 5 μg/ml human transferrin, 5 × 10−7 M T3, 5 × 10−10 M hydrocortisone, 25 ng/ml PGE1, and 5 × 10−6 M selenium. MDCK stock cultures were subcultured using 0.53 mM EDTA and 0.05% trypsin in phosphate-buffered saline, followed by inhibition of trypsin action with soybean trypsin inhibitor as described previously (17). MDCK cells were to be utilized for transient transfection studies were plated at 2 × 104 cells/35-mm dish into dishes containing DMEM/ Ham’s nutrient mixture F-12 supplemented with 5 μg/ml insulin and 5 μg/ml transferrin utilizing Lipofectamine. The next day, the medium was changed to DMEM/nutrient mixture F-12 supplemented with 5 μg/ml insulin and 5 μg/ml transferrin, and the cultures were returned to a 5% CO2 and 95% air humidified environment. Two hours later, appropriate effector molecules were added to the cultures, and the cultures were then allowed to incubate for 4 h (unless indicated otherwise). At the end of the incubation, the monolayers were solubilized in reporter lysis buffer. After the cell lysate was centrifuged for 10 min, the supernatant was utilized for assays of luciferase and β-galactosidase activities or stored at −20 °C until used.

To measure luciferase activity, an aliquot of the cell lysate was placed in 20 μl of luciferase assay buffer containing 20 μl Tricine, 1.07 mM MgCO3, 4.07 mM MgSO4, 0.1 mM EDTA, 33.3 μM dithiothreitol, 270 μM CoA, 470 μM luciferin, and 500 μM ATP. The light units were determined using a Packard Tri-Carb 4520 scintillation counter with the coincidence circuit turned off. To determine the β-galactosidase activity of the transfected cells, aliquots of cell extracts were incubated at 23 °C in reaction buffer containing the Galacton-Star® substrate (provided in the Galac-to-Star™ system), and the light emitted was then measured as described above. β-Galactosidase activity is expressed as a fraction of the activity in control transfected cultures.

Each determination of luciferase activity was normalized with respect to the β-galactosidase activity of the culture. The normalized luciferase value obtained in each condition was the mean ± S.E. of quadruplicate determinations. The percent normalized value was compared with the indicated control value from the same experimental culture set. The significance of each observed stimulatory (or inhibitory) effect was determined by one-way analysis of variance and the Newman-Keuls multiple comparison test (using programs in Prism 4 software). Differences were determined as being significant when p < 0.05.

**Plasmid Construction**—Previously, the human Na,K-ATPase β1 subunit gene promoter was inserted into the pOLuc plasmid (to obtain the construct pHj1–1141Luc) (12). A set of deletion mutants within the human Na,K-ATPase β1 subunit promoter (pHj1–128Luc, pHj1–554Luc, pHj1–456Luc, pHj1–327Luc, and pHj1–83Luc) was constructed as described previously by Feng et al. (12). Subsequently, we prepared additional deletion mutants (pHj1–212Luc, pHj1–232Luc, and pHj1–182Luc) by exonuclease III digestion of pHj1–554Luc following cleavage of pHj1–554Luc at the unique SalI site (at the SphI site). The SphI-BstN sites, located immediately 5′ relative to the β1 subunit promoter region.

A double-stranded synthetic oligodeoxynucleotide with a sequence homologous to −167 to −72 of the human Na,K-ATPase β1 promoter, AGCTCTCA AGGGCGCCCT TCTGCGCCG CGGCTCTTCT GTGC- CGCCCG CGAAACCCGGG CTCCTGGGCG CAGTCCTCGT CCTGCGCC GCAGGATTGCG (with the GC boxes underlined and the PORE boldface and underlined), was ligated into the HindIII/Xhol sites on the
polylinker of the pLuc-MCS plasmid upstream from the TATA box, creating the expression vector pLuc-MCS-β2−β2. In addition, a synthetic oligodeoxynucleotide in which the two GC boxes were translocated as indicated with a AGCTGCGGCGGGCGCCCTCTAGCCCGCCGGCTTTGGCCTGGGGTCGGGGGCGGGC GGACAGCCCGGCCC. The samples obtained from immunoprecipitations and GST pull-down assays were separated by SDS-PAGE using either 12% gels for Sp1 sites of pLuc-MCS, creating the expression vector pLuc-MCS-PGREFN. The cultures were treated for 4 h with 500 ng/ml PGE1. Subsequently, the cultures were lysed in buffer containing 20 mM HEPES (pH 8.0), 120 mM NaCl, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonil fluoride, 1 mM dithiothreitol, and 0.5 mM leupeptin. After mixing by rotation with rabbit anti-CREB or rabbit anti-Sp1 antibody coupled to protein G-Sepharose in lysis buffer and then washed by centrifugation in a microcentrifuge at 4,000 rpm for 5 min. Immunoprecipitations and GST pull-down assays were conducted with the pβ2−1141Luc reporter plasmid, containing the human full-length Na,K-ATPase β2 subunit promoter (−1141 to +490), and pSβgal by the Lipofectamine method. Twenty-four hours later, the cells were treated with either 500 ng/ml PGE1, or 1 mM 8-Br-cAMP for 4 h, followed by assays of cell extracts for luciferase activity. Values are the mean luciferase activity (light units) ± S.D. of four determinations and were normalized with respect to β-galactosidase activity.

**RESULTS**

**Effect of PGE1 and 8-Br-cAMP**—To study the regulation of the human Na,K-ATPase β2 promoter activity by PGE1 and 8-Br-cAMP, MDCK cells were cotransfected with the pHβ1−1141Luc reporter plasmid, containing the human full-length Na,K-ATPase β2 subunit promoter (−1141 to +490), and pSβgal by the Lipofectamine method. Twenty-four hours later, the cells were treated with either 500 ng/ml PGE1, or 1 mM 8-Br-cAMP, or no further supplement (control). Fig. 1 shows that PGE1 caused a 4.8 ± 0.3-fold increase in luciferase activity relative to the control, whereas 8-Br-cAMP caused a 4.4 ± 0.4-fold increase. Previously, both a mineralocorticoid/glucocorticoid-responsive element (MRE/GRE; −572 to −552) (24) and a thyroid hormone-responsive element (TRE; −459 to −438) (12) were identified on the human Na,K-ATPase β2 subunit promoter (Fig. 2A). To determine whether either of these elements is required for PGE1 or 8-Br-cAMP stimulation, transient transfection studies were conducted with the 5′-deletion constructs

**Electrophoretic Mobility Shift Assays**—The custom-synthesized double-stranded synthetic oligonucleotides were 32P-labeled by the random priming method using [α-32P]dCTP. The consensus CRE oligonucleotide, the mutant CRE oligonucleotide, and the consensus Sp1 site were 32P-labeled when used as probes. Unlabeled double-stranded oligonucleotides were also used as competitors. Included among the oligonucleotide used were the PGRE (TGCTGGGCGCCGAGCTCGG-CCCTGGCGCCG, −81 to −111), the synthetic PGRE (SPGRE) (GGCTCCGACCCTCCCTGGCGCCGAGCTCGGGCCCTGGCGGC- GCGC, −72 to −102), the consensus CRE (AGAGAGACCTCGAG), a mutant CRE (AGAGAGACCTCGAG), and the consensus Sp1 oligonucleotide (ATAGATCGATGGGCGGGCGG).

**Stimulation of Na,K-ATPase**

**FIG. 1. Stimulation of Na,K-ATPase β2 subunit promoter activity by PGE1, and 8-Br-cAMP.** MDCK cells were cotransfected with the pHβ1−1141Luc reporter plasmid, containing the human full-length Na,K-ATPase β2 subunit promoter (−1141 to +490), and pSβgal by the Lipofectamine method. Twenty-four hours later, the cells were treated with either 500 ng/ml PGE1, or 1 mM 8-Br-cAMP for 4 h, followed by assays of cell extracts for luciferase activity. Values are the mean luciferase activity (light units) ± S.D. of four determinations and were normalized with respect to β-galactosidase activity.

**Effect of PGE1 and 8-Br-cAMP**—To study the regulation of the human Na,K-ATPase β2 promoter activity by PGE1 and 8-Br-cAMP, MDCK cells were transiently transfected with the pHβ1−1141Luc, a human Na,K-ATPase β2 promoter/luciferase construct (12). Subsequently, the cultures were incubated for 4 h in the presence of 500 ng/ml PGE1, or 1 mM 8-Br-cAMP, or no further supplement (control). Fig. 1 shows that PGE1 caused a 4.8 ± 0.3-fold increase in luciferase activity relative to the control, whereas 8-Br-cAMP caused a 4.4 ± 0.4-fold increase. Previously, both a mineralocorticoid/glucocorticoid-responsive element (MRE/GRE; −572 to −552) (24) and a thyroid hormone-responsive element (TRE; −459 to −438) (12) were identified on the human Na,K-ATPase β2 subunit promoter (Fig. 2A). To determine whether either of these elements is required for PGE1 or 8-Br-cAMP stimulation, transient transfection studies were conducted with the 5′-deletion constructs

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shown in Fig. 2A. Fig. 2 (B and C) indicates that the stimulatory effects of PGE$_1$ and 8-Br-cAMP were retained in deletion constructs lacking the MRE/GRE (located at −572 to −552; as exemplified by the deletion mutant pH$\beta_1$–554Luc) and/or the TRE (located at −459 to −438; as exemplified by the deletion mutant pH$\beta_1$–327Luc).

Stimulation by PGE$_1$ and 8-Br-cAMP was even retained in the deletion mutant pH$\beta_1$–182Luc. However, in the deletion mutant pH$\beta_1$–83Luc, activation of luciferase activity by PGE$_1$ and 8-Br-cAMP was lost completely. These results suggest that a regulatory element(s) located between −182 and −83 is required to elicit the stimulatory effects of PGE$_1$ and 8-Br-cAMP.

Electrophoretic Mobility Shift Assays—A putative response element (a PGRE) was identified (AGTCCCTGCG) that was located between −98 and −92 and that was within the region of the $\beta_1$ promoter required to observe the stimulatory effects of PGE$_1$ and 8-Br-cAMP (i.e. from −182 to −82) (Fig. 2A). To assess the ability of this site to bind nuclear proteins, electrophoretic mobility shift assays were conducted with a $^{32}$P-labeled synthetic oligonucleotide probe (CTCGGCGCCGAGTC-CCTGCCAGCAGAGCTAG) homologous to −111 to −81 within the $\beta_1$ subunit promoter, a region that contains the putative PGRE (−98 to −92). The labeled oligonucleotide was incubated with nuclear extracts from MDCK cells and then subjected to electrophoresis. Fig. 3A shows the presence of retarded $^{32}$P-labeled bands, protein-DNA complexes that formed when the PGRE was used as a probe. Because the PGRE has sequence similarity to a consensus CRE, the effect of the addition of a competing consensus CRE oligonucleotide (AGAGATTGCCTGTGGTCA) was examined. When this competing unlabeled consensus CRE oligonucleotide was included in the binding reaction at a 200-fold excess, the intensity of four of the labeled bands was reduced (by 40% (band A), 35% (band B), 39% (band C), and 25% (band D)). Although this inhibition was considerable, it was not to the extent observed when the PGRE itself was used as a competitor (100% inhibition obtained at 200-fold excess).

Fig. 3B shows the presence of two retarded $^{32}$P-labeled bands following electrophoresis when the same consensus CRE was used as a $^{32}$P-labeled probe during the binding reaction. The intensity of the two labeled bands was partially reduced (by 2-fold (band A) and 1.5-fold (band B)) when a 200-fold excess of the unlabeled PGRE oligonucleotide was used as a competitor. In contrast, the intensity of the bands was reduced to essentially zero by a 200-fold excess of the unlabeled consensus CRE itself. Thus, these results indicate that the consensus CRE is a more effector competitor of the $^{32}$P-labeled CRE probe than the PGRE. However, when an AC → TG mutation was introduced into the unlabeled CRE (AGAGATTGCGTGGTCAAGAGCTAG), and this CRE was used as a competitor of the $^{32}$P-labeled CRE, the intensity of the two labeled bands was only partially reduced (by 1.6-fold (band A) and 1.2-fold (band B)). This result indicates that the mutant CRE is a less competitive competitor than the PGRE.

If the PGRE could effectively act as a CRE, then a CREB probe would be expected to be a component of a nuclear protein-PGRE binding complex. This possibility was evaluated by incubating an antibody against CREB with the $^{32}$P-labeled PGRE and the nuclear protein preparation, followed by electrophoresis. As shown in Fig. 3C, a supershift was observed in the presence of anti-CREB antibody, unlike with the control nuclear protein-PGRE binding complex. Fig. 4C also shows that a similar supershift was obtained with anti-CREB antibody when a $^{32}$P-labeled consensus CRE oligonucleotide was used as a probe, unlike with the control using preimmune serum (or IgG).
Another possible component of such a nuclear protein-PGRE binding complex is the CBP. Fig. 3D shows that when an antibody against CBP was added to the binding reaction, the antibody did indeed act as a competitor, reducing the formation of nuclear protein-PGRE complexes by 58% when added at 6 μg/ml.

The region of the β1 promoter surrounding the putative PGRE is GC-rich and even contains a consensus Sp1 site (Fig. 2A). To examine the possibility that these GC-rich sites could be involved in the formation of the nuclear protein-DNA complex, which occurs in this region of the promoter, binding studies were conducted using a longer synthetic oligonucleotide (SPGRE) homologous to region −125 to −82 of the β1 promoter (GCCTCCCTGCCCCCTGGCCCGAGTCCCTGCCCCCTGGCCCG). The additional nucleotides present in this SPGRE (double-underlined) contained a consensus Sp1 site (−116 to −112) as does a downstream region including the PGRE itself (also double-underlined) (25).

**Fig. 3.** Binding of nuclear proteins to a PGRE and a consensus CRE oligonucleotide. A, a 32P-labeled PGRE oligodeoxynucleotide probe (−111 to −81) containing a putative PGRE was incubated with nuclear extracts from MDCK cells for 30 min as described under “Experimental Procedures.” To study the effect of a competitor, a 200-fold excess of either an unlabeled PGRE or an unlabeled CRE was added simultaneously with the labeled probe at the start of the binding reaction. All lanes received 6 μg of protein. B, a 32P-labeled consensus CRE was incubated with MDCK cell nuclear extracts. In competition studies, a 200-fold excess of an unlabeled PGRE, a CRE, or a mutant CRE was added to the binding reaction. C, rabbit anti-CREB polyclonal antibody (11 μg) was incubated with nuclear extracts from MDCK cells 10 min prior to the addition of either a 32P-labeled PGRE oligonucleotide probe or a 32P-labeled consensus CRE oligonucleotide probe. Comparisons were made with either the control binding reaction without antibody or the control binding reaction with an equivalent amount of rabbit IgG. D, anti-CBP polyclonal antibody (2, 4, 6, or 8 μg) was incubated with nuclear extracts from MDCK cells 10 min prior to the addition of 32P-labeled PGRE to the binding reaction, and electrophoretic migration was compared with control nuclear extracts without antibody.
oligonucleotide was not as effective a competitor as either the consensus Sp1 oligonucleotide or the SPGRE itself. (The consensus CRE caused only a 23% decrease in band A, whereas the consensus Sp1 oligonucleotide and the SPGRE oligonucleotide caused 91 and 98% decreases, respectively, in the intensity of band A.)

As the gel retardation studies suggested that Sp1 bound to the SPGRE, the effect of anti-Sp1 antibody on nuclear protein binding to a 32P-labeled SPGRE probe was examined. Fig. 4C shows supershift in the presence of anti-Sp1 antibody, unlike with the controls either without antibody or with preimmune serum. Similar results were obtained when a 32P-labeled PGRE probe was used.

To evaluate further the involvement of Sp1 and CREB, gel retardation studies were conducted using either a 32P-labeled consensus Sp1 or CRE oligonucleotide (as described above). Fig. 4D shows that an unlabeled PGRE oligonucleotide was an effective competitor of nuclear complex formation with the 32P-labeled consensus Sp1 probe (causing a 66% reduction in the intensity of band A and an 81% reduction in the intensity of band B). Similarly, when a 32P-labeled consensus CRE probe was used, the unlabeled PGRE oligonucleotide reduced binding by 46% (band A) and 36% (band B). The unlabeled SPGRE was equally effective as a competitor, although not to the extent observed when the PGRE was used as a competitor (Fig. 4B).

Role of the PGRE and Sp1 Sites in Mediating PGE1 Stimulation—The results obtained above suggested that the Sp1 sites in the human β1 subunit promoter adjacent to the PGRE are involved in regulation by PGE1. To examine further the role of the PGRE and Sp1 sites in regulation by PGE1, transient
transfection studies were conducted with MDCK cells using the vector pLuc-MCS, which contains a minimal promoter with a TATA box, as well as the recombinant vector pLuc-MCS-FN, which contains region 118 to 36 of the human FN promoter with four GC boxes (Fig. 5A) immediately upstream from the TATA box in pLuc-MCS. Fig. 5C shows a 5.2 ± 1.4-fold stimulation by PGE1 in MDCK cells transfected with pLuc-MCS-FN as compared with a 1.7 ± 0.6-fold stimulation observed in cultures transfected with pLuc-MCS. PGE1 stimulation was even greater (57 ± 19-fold) in the pLuc-MCS-FN-PGRE vector, which contains the PGRE (CGAGTCCCTGC) in region 167 to 72 of the FN promoter. The results thus show 1) that a PGE1 response could be observed in a heterologous promoter and 2) that both the PGRE and Sp1 sites were involved in mediating PGE1 stimulation mediated by this alternative promoter.

To examine further the involvement of the PGRE and Sp1 sites in the β1 subunit promoter, the effect of PGE1 was also examined in MDCK cells transiently transfected with pLuc-MCS containing region the −167 to −72 of the human β1 subunit promoter (pLuc-MCS-β22–167) (Fig. 5B). Fig. 5C shows that the PGE1 stimulation was 25 ± 4-fold in MDCK cells transiently transfected with pLuc-MCS-β22–167 compared with the basal luciferase expression level obtained with pLuc-MCS in the absence of PGE1. Region −167 to −72 contains two GC boxes immediately upstream from the PGRE (Fig. 5A). Fig. 5C shows that when the two GC boxes in region −167 to −72 were translocated farther upstream from the PGRE (as in pLuc-MCS-β22–167-2GCtrans), the PGE1 stimulation was reduced to only 1.3 ± 0.3-fold of the level observed in MDCK cells transfected with pLuc-MCS in the absence of PGE1. This latter result suggests that the proximity of the Sp1 sites to the PGRE in the β1 promoter is a critical factor in determining the extent of the PGE1 stimulation.

Interaction between CREB and Sp1—As the results described above were suggestive of an interaction between Sp1
and CREB, the possibility was examined that CREB and Sp1 could be co-immunoprecipitated from MDCK cells. To this end, immunoprecipitation studies were conducted with a lysate obtained from PGE1-treated MDCK cells using antibody against Sp1, CBP, or CREB. The immunoprecipitates were separated by SDS-PAGE and transferred to nitrocellulose.

Fig. 6A shows the results of a Western analysis with anti-CREB antibody. In the lanes of the gel containing the samples from the Sp1 and CBP immunoprecipitates, bands that co-migrated with immunoprecipitated CREB were observed. Similarly, when examining the results of an analysis with anti-Sp1 antibody (Fig. 6B), the lanes containing the samples from the CREB and CBP immunoprecipitates were observed to possess bands that co-migrated with immunoprecipitated Sp1. Fig. 6B also shows the results of a GST pull-down assay using GST-CREM. The GST-CREM pull-down similarly contained protein bands that co-migrated with Sp1, unlike the case with the GST pull-down.

Role of CREB, Sp1, and Histone Acetyltransferase in Mediating PGE1 Stimulation—To determine whether CREB is indeed involved in mediating PGE1 stimulation observed with pHβ1−1141Luc, the effect of dominant-negative CREB (KCREB) (14) on the PGE1 response was examined. Fig. 7A shows that in MDCK monolayers cotransfected with pHβ1−1141Luc and KCREB, PGE1 stimulation was reduced by 65 ± 8% (i.e. 2.8 ± 0.6-fold PGE1 stimulation in MDCK cells with KCREB compared with 8.0 ± 1.7-fold in MDCK cells with the empty vector). KCREB did not have a similar inhibitory effect in MDCK cell cultures maintained in the absence of PGE1.

To evaluate the influence of Sp1, MDCK cells were cotransfected with pHβ1−1141Luc and pcDNA3-Sp1, which encodes full-length Sp1 (13). Fig. 7B shows that the PGE1 stimulation increased significantly in the presence of pcDNA3-Sp1 (1.7-fold) compared with the PGE1 stimulation observed in MDCK cells cotransfected with pHβ1−1141Luc and the empty vector (pcDNA3). To evaluate further the possible involvement of Sp1 in the PGE1 stimulation, the effect of mithramycin A, an anti-
Inhibition of PGE$_1$ stimulation by mithramycin A

MDCK cells were plated into 35-mm dishes containing 5 μg/ml insulin and 5 μg/ml transferrin at 30° cells/dish. The day after plating, the monolayers were transfected with pHβ1–1141Luc and pSVβgal as described under “Experimental Procedures.” Two hours after transfection, 200 nM mithramycin A was added to part of the cultures. The subsequent day, monolayers were incubated for 4 h with (a) insulin and transferrin alone (control); (b) the control + 500 ng/ml PGE$_1$; (c) the control + 200 nM mithramycin A, or (d) the control + 500 ng/ml PGE$_1$ and 200 nM mithramycin A. At the end of the incubation, the luciferase activity was determined and standardized with respect to β-galactosidase activity. Values are means ± S.E. of five determinations. The values were analyzed by one-way analysis of variance. The values obtained with cultures incubated in the presence of PGE$_1$ and mithramycin A differed significantly from the control value (p < 0.05). The values obtained with cultures incubated in the presence of PGE$_1$ and mithramycin A also differed significantly from the values obtained in the presence of PGE$_1$, 6.2 ± 0.5.

| Effector molecule | Stimulation (fold) |
|-------------------|--------------------|
| Control           | 1.0 ± 0.3          |
| +PGE$_1$          | 20.2 ± 2.0         |
| +Mithramycin A    | 1.0 ± 0.1          |
| +PGE$_1$ and mithramycin A | 7.3 ± 1.1 |

Table 1

Biotic that has a GC-specific binding affinity for DNA (26), was determined. Table I shows that mithramycin A reduced the PGE$_1$ stimulation by 64 ± 5%.

Following CREB phosphorylation, CREB interacts with CBP, which in turn regulates transcription through its histone acetyltransferase activity (16). To determine whether histone acetylation or deacetylation is an aspect of the PGE$_1$ stimulation, the effect of trichostatin, a histone deacetylase inhibitor, was examined. Fig. 7C shows that trichostatin reduced the PGE$_1$ stimulation by 66 ± 4% to 4.0 ± 0.5-fold compared with cultures treated with PGE$_1$ alone (11.5 ± 0.9-fold). However, Fig. 7B also shows that anacardic acid, a histone acetylase inhibitor, also had an inhibitory effect on the PGE$_1$ stimulation.

Involvement of Other Signaling Pathways in Mediating the PGE$_1$ Response—PGE$_1$, has been observed to cause an increase in intracellular calcium levels as well as intracellular cAMP levels in MDCK cells (27, 28). Thus, PGE$_1$ may initiate signaling through PKC and/or Ca$^{2+}$/calmodulin-dependent protein kinase (CaM kinase) as well as cAMP-dependent protein kinase. To evaluate the former possibility, the effect of PMA was examined. Fig. 8A shows that a 2.5-fold stimulation by 10$^{-9}$ M PMA in pHβ1–1141Luc. Stimulation by PMA was maintained in deletion mutants ranging from pHβ1–554Luc to pHβ1–182Luc. However, the stimulatory effect of PMA was not observed in pHβ1–83Luc, suggesting that, as was the case with PGE$_1$, PMA stimulation was dependent upon region –182 to –83. As a control in this experiment, the inactive analog 4x-PMA was examined (Table II) and was found to have no significant effect on transcription by pHβ1–1141Luc.

PMA, 8-Br-cAMP, and PGE$_1$ may stimulate transcription by pHβ1–1141Luc by either the same or a different mechanism. To determine whether CREB activation is involved in mediating the stimulatory effects of 8-Br-cAMP, PMA, and PGE$_1$, MDCK cells were cotransfected with the pFR-Luc reporter plasmid and either the transactivator plasmid pFA2-CREB or pFC2-db1 (a negative control). The transactivator plasmid pFA2-CREB encodes a transactivator protein containing the CREB activation domain linked to the yeast GAL4 DNA-binding domain. When the CREB activation domain is phosphorylated, the fusion protein binds to the GAL4-binding sites on pFR-Luc, stimulating luciferase gene transcription. Fig. 8B shows that 10$^{-7}$ M PMA and 500 ng/ml PGE$_1$ stimulated luciferase gene expression by the pFR-Luc reporter plasmid by 96 ± 12- and 494 ± 113-fold, respectively (compared with luciferase gene expression obtained with the pFC2-db1 plasmid in the presence of PGE$_1$). Fig. 8C shows that in parallel cultures, PMA and PGE$_1$ stimulated transcription by pHβ1–1141Luc by 1.8 ± 0.1- and 8.5 ± 0.3-fold, respectively. Similarly, Fig. 8D shows a trans-stimulation in response to 1 μM 8-Br-cAMP (51 ± 1-fold), albeit not to the extent obtained in parallel cultures maintained with PGE$_1$ (253 ± 50-fold).

To evaluate the possible involvement of CaM kinase, the effect of two inhibitors of CaM kinase II, KN-62, and KN-93 (29), on the PGE$_1$ stimulation of pHβ1–1141Luc was examined. Fig. 9A shows that 3 μM KN-62 and 3 μM KN-93 each individually reduced the PGE$_1$ stimulation by 63 ± 3 and 60 ± 2%, respectively. In contrast, KN-92, an inactive analog of KN-93, was not inhibitory. The ability of KN-62 to inhibit the PGE$_1$ stimulation of the constructs containing 5'-deletions in the β$_1$ subunit promoter was examined. Fig. 9B shows that 3.3 μM KN-62 partially inhibited the PGE$_1$ stimulation not only of pHβ1–1141Luc, but also of 5'-deletion constructs ranging from pHβ1–554Luc to pHβ1–182Luc. Thus, the component of the PGE$_1$ stimulation remaining in the presence of KN-62 is still dependent upon region –82 to –182.

CaM kinase II is dephosphorylated and deactivated by okadaic acid-sensitive type I and type 2A protein phosphatases (30, 31). For this reason, okadaic acid is a reported activator of CaM kinase II. Thus, the effect of 5 × 10$^{-7}$ M okadaic acid on transcription was examined in pHβ1–1141Luc as well as in constructs containing 5'-deletion mutations. Fig. 9C shows that okadaic acid caused a 3-fold stimulation of luciferase activity not only in pHβ1–1141Luc, but also in 5'-deletion mutants ranging from pHβ1–554Luc to pHβ1–182Luc. Thus, the component of the PGE$_1$ stimulation remaining in the presence of KN-62 is still dependent upon region –82 to –182.

DISCUSSION

Prostaglandins are potent regulators of ion transport in the kidney, affecting the activity of transport systems, including the Na,K-ATPase (10). Despite the physiologic importance of these effects, little is known about the mechanisms by which renal prostaglandins regulate the activity of membrane transport systems. Due to the diverse effects of prostaglandins on the different nephron segments in the kidney, many previous studies have been conducted with isolated nephron segments, placing limitations on mechanistic studies (10). These limitations can be overcome to a large extent by the use of established kidney tubule epithelial cell lines.

In this study, the MDCK cell line, a model of kidney distal tubule epithelial cells, has been used to study regulation of the Na,K-ATPase β$_1$ subunit gene. The Na,K-ATPase is a heterodimer consisting of an α as well as a β subunit (32). However, unlike the α subunit, the β subunit has been reported to be a limiting factor in heterodimer formation (33, 34). Indeed, in a number of tissues, overexpression of the β subunit relative to the α subunit in response to particular effector molecules is associated with an increase in Na$^+$ pump activity (35). Thus, in this study, PGE$_1$ effects on the transcription of the Na,K-ATPase β$_1$ subunit gene in MDCK cells have been examined. To identify a region in the β$_1$ promoter required for regulation by PGE$_1$, transient transfection studies were conducted using β$_1$ promoter/luciferase constructs containing 5'-deletions in the β$_1$ promoter fused to the luciferase gene. Using this approach, we identified a region in the promoter (–182 to –83) that is required for regulation by PGE$_1$, 8-Br-cAMP, and Ca$^{2+}$. Our electrophoretic mobility shift studies indicated that DNA within this region (located between –100 and –92) is capable of binding to CREB as well as to Sp1.

![Graph](http://www.jbc.org/)
Previously, pH\textsubscript{β1} subunit promoter with 5′-deletions of varying lengths (including −1141, −554, −327, −312, −232, −182, and −83) and pSV\textsubscript{gal} was utilized to define a TRE (−1100 to −1141) Luc and 5′-deletion mutants were utilized to define a TRE (−1100 to −1141) present within the human Na,K-ATPase β1 subunit promoter (12), in addition to a MRE/GRE (−650 to −630) (24). Our transient transfection studies indicated that PGE\textsubscript{1}, 8-Br-cAMP, and calcium all stimulated transcription. Using constructs containing 5′-deletions within the β1 subunit promoter fused to luciferase, we located a region distinct from the TRE and MRE/GRE, located between −182 and −83 in the β1 subunit promoter, that is required to elicit the effects of PGE\textsubscript{1}, cAMP, and calcium. We identified a sequence within this region (−100 to −92), a β1 PGRE, that contains a putative CRE as well as an Sp1-binding site. Although neither the MRE/GRE nor the TRE was required to elicit the effects of PGE\textsubscript{1}, cAMP, and calcium, we cannot rule out the possibility of synergistic interactions between thyroid hormone, glucocorticoids, and prostaglandins. Indeed, all of these factors regulate the growth of MDCK cells in hormonally defined serum-free medium (17).

PGE\textsubscript{1} has previously been reported to activate both the cAMP and Ca\textsuperscript{2+} pathways in MDCK cells. Both cAMP and calcium may regulate transcription through the phosphorylation of CREB (36). Not only have both isoforms of the catalytic subunit of cAMP-dependent protein kinase been reported to phosphorylate CREB at Ser\textsuperscript{133} (37), but in addition, PKC and
Oxidative stress is mediated through increased Sp1 binding (48). Similarly, CaM kinase have also been reported to phosphorylate CREB (36, 38). The phosphorylation of CREB at Ser133 induces binding of CREB to CBP and increases the ability of CREB to bind to CREs located in promoters for genes such as prolactin (37), c-fos (39), and somatostatin (40). In addition, nuclear Ca2+ and CaM kinase IV have been reported to regulate CREB-mediated transcription through their effects on the CREB coactivator CBP (41, 42). Ultimately, the activated CBP nuclear protein-CRE complex binds to a regulatory element. In some cases, the cAMP-dependent protein kinase- and PKC-mediated signaling pathways converge so as to modulate gene expression through a common cis-acting element, a CRE (43). In other cases, agents such as PMA, which activate PKC, stimulate transcription through a separate cis-acting element, a TRE (or phorbol ester-responsive element) (44, 45). The results of our studies are consistent with the presence of a single cis-acting element. PGRE, which is responsible for transcriptional control of the β1 subunit gene by the cAMP and PKC pathways.

To determine whether either a CRE or CREB may possibly be involved in regulation by PGE1, cAMP, and Ca2+ -mediated pathways, electrophoretic mobility shift assays were conducted. The results of our studies indicate that four protein-DNA complexes formed between a synthetic oligonucleotide containing a putative β1 PGRE. Not only was a consensus CRE able to compete with a 32P-labeled β1 PGRE, but in addition, a supershift was observed when a polyclonal antibody against CREB was present during the binding reaction. Our results also indicate that the β1 PGRE similarly acted as a competitor against a 32P-labeled CRE probe, although the β1 PGRE was less effective as a competitor than an unlabeled CRE.

Our electrophoretic mobility shift studies indicated that a synthetic oligodeoxynucleotide containing a consensus Sp1-binding site (i.e., a GC box) was able to compete with the 32P-labeled β1 PGRE. Moreover, a supershift of 32P-labeled protein-β1 PGRE complexes was observed when a polyclonal antibody against Sp1 was included in the binding reaction. Sp1 is the first identified member of the zinc finger transcription factor family that binds to the core sequence GG(C/T/A)GG (46) present on a large number of promoters. Although Sp1 family members often function to maintain basal transcription rates, Sp1 also plays an important role in regulation of transcription under conditions such as oxidative stress (47). Indeed, in two lung epithelial cell lines and in MDCK cells, the increased transcription of the rat β1 subunit gene in response to hyperoxia is mediated through increased Sp1 binding (48). Similarly, in neonatal rat cardiac myocytes, the increased rat β1 subunit transcription rate is associated with increased Sp1 binding (49). Increased transcription rates observed as a consequence of Sp1 action may be the consequence of its phosphorylation as well as its interaction with Sp3 (48, 49).

| Effector molecule | Stimulation |
|-------------------|-------------|
| Control           | 1.00 ± 0.06 |
| +500 ng/ml PGE1   | 37.9 ± 4.5  |
| +10⁻⁹ M 4α-PMA    | 0.88 ± 0.29 |
| +10⁻⁹ M PMA       | 4.1 ± 0.72  |

*CaM kinase has also been reported to phosphorylate CREB (36, 38). The phosphorylation of CREB at Ser133 induces binding of CREB to CBP and increases the ability of CREB to bind to CREs located in promoters for genes such as prolactin (37), c-fos (39), and somatostatin (40). In addition, nuclear Ca2+ and CaM kinase IV have been reported to regulate CREB-mediated transcription through their effects on the CREB coactivator CBP (41, 42). Ultimately, the activated CBP nuclear protein-CRE complex binds to a regulatory element. In some cases, the cAMP-dependent protein kinase- and PKC-mediated signaling pathways converge so as to modulate gene expression through a common cis-acting element, a CRE (43). In other cases, agents such as PMA, which activate PKC, stimulate transcription through a separate cis-acting element, a TRE (or phorbol ester-responsive element) (44, 45). The results of our studies are consistent with the presence of a single cis-acting element, a PGRE, which is responsible for transcriptional control of the β1 subunit gene by the cAMP and PKC pathways.

Our results indicate that regulation of transcription of the human β1 subunit gene by the cAMP and Ca2+ pathways involves an interaction between Sp1, CREB, and a regulatory region containing adjacent Sp1 and PGRE sites. Consistent
with an interaction between Sp1 and CREB are our immunoprecipitation experiments indicating that Sp1 could be co-immunoprecipitated with CREB and CBP. In addition, our results indicate that GST-CREM could pull-down Sp1. Also consistent with the involvement of CREB and Sp1 in regulation by PGE$_1$ are our observations that cotransfection of pH$_{1141}$Luc with an expression vector encoding KCREB (a dominant-negative CREB) resulted in reduced stimulation by PGE$_1$, whereas cotransfection with an expression vector encoding full-length Sp1 resulted in an increase in PGE$_1$ stimulation.

Similarly, Kobayashi and Kawakami (50) found evidence for an activating transcription factor (ATF)/CRE site (−70 to −63) and an adjacent GC box (−57 to −48) in proximity to a TATA-like sequence (−33 to −27) in the rat a$_1$ subunit promoter. Evidence for similar sequences in the human a$_1$ subunit promoter was also presented by these same investigators. They introduced point mutations at either the ATF/CRE site or the adjacent GC box that resulted in a dramatic reduction in promoter activity. Thus, they concluded that both of these sites are essential for efficient constitutive transcription through a synergistic interaction between transcription factors (50).

Unlike the studies of Kobayashi and Kawakami (50), this study presents evidence indicating that regulation by cAMP, Ca$^{2+}$, and PGE$_1$ requires the presence of a site that is composed of a CRE as well Sp1. Indeed, we observed that the pLuc-MCS-FN vector, which contains four GC boxes in the promoter region, was responsive to regulation by PGE$_1$. However, insertion of the PGRE between the four GC boxes of the human FN promoter in the pLuc-MCS-FN vector resulted in an increased responsiveness to PGE$_1$ by MDCK cells, suggestive of a synergism between the Sp1 sites and the PGRE in the FN promoter. Further evidence was obtained for the involvement of Sp1 in mediating the PGE$_1$ response from the transient transfection studies conducted with the pLuc-MCS-β72−167 vector, which contains region −167 to −72 in the β$_1$ subunit promoter. Although the PGE$_1$ stimulation was observed in MDCK cells transfected with pLuc-MCS-β72−167, it was attenuated when the two GC boxes immediately adjacent to the PGRE were moved farther upstream from the PGRE.

Evidence for the involvement of Sp1 in regulation by the cAMP- and PKC-mediated pathways has been reported previously. Indeed, in the murine 8S-lipoxygenase gene, the phorbol ester-responsive element (a TRE) was found to be a binding site for Sp1 (26), and increased Sp1 binding to the TRE was observed following PMA treatment. Similarly, activation of the apolipoprotein A-I gene in HepG2 cells by the cAMP-dependent protein kinase and PKC pathways has been shown to be dependent upon an insulin-responsive core element, which is actually a consensus binding site for Sp1 (51). In a similar manner, evidence has been presented indicating that the binding of Sp1 to a consensus Sp1 site on the rat mitochondrial serine:pyruvate aminotransferase promoter is involved in the cAMP-dependent protein kinase-mediated expression of the rat serine:pyruvate aminotransferase gene (52).

Both CREB and Sp1 have been implicated in the transcriptional control of the loricrin gene in cultured human keratinocytes (53). The increased loricrin transcription observed in advanced differentiated cells was attributed to synergistic interactions between Sp1, c-Jun, and p300. Activation of the cAMP pathway in the keratinocytes resulted in increased translocation of Sp1 from the cytoplasm to the nucleus, contributing to the observed increase in loricrin gene transcription. However, activation of CREB was observed to inhibit transcription, presumably due to CREB binding to a CRE adjacent to a KSSE site, which was immediately downstream from the Sp1 site. In advanced differentiated cells, CREB protein levels were found to decline, presumably permitting transcription of the loricrin gene to proceed.

Our results with the human β$_1$ subunit promoter differ from the results obtained in the reports described above in that we obtained evidence for the involvement of CREB in addition to Sp1 in mediating the stimulatory effects of the cAMP-dependent protein kinase, PKC, and CaM kinase pathways on β$_1$ gene transcription. Our results suggest that an interaction between Sp1 and CREB is involved in regulation of β$_1$ subunit gene expression through these pathways. Such an interaction between Sp1 and CREB may be either direct or indirect. Consistent with an indirect interaction are reports indicating that both Sp1 and CREB interact with CBP as well as transcription factor IID (16).

Although the involvement of both Sp1 and CREB in regulation by the cAMP-dependent protein kinase pathway has been indicated previously, evidence was not presented for the involvement of a protein complex involving Sp1 and CREB. Included among previously published studies is a report concerning the regulation of the gene encoding CREB itself by cAMP in rat Sertoli cells, involving the binding of endogenous CREB to two CREs on the CREB promoter as well as the binding of Sp1 to an adjacent Sp1 site (54). Transcriptional regulation of the gene encoding chromogranin A in enterochromaffin-like cells by gastrin, PMA, and dibutyryl cAMP has also been found to be dependent upon an Sp1/Egr-1 site that is in close proximity to a CRE (55). Thus, in these and other experimental systems, interactions between CREB and Sp1 may be involved in gene regulation.

Whether or not the Na,K-ATPase α and β subunit genes are regulated in a parallel manner by PGE$_1$-, cAMP-, and Ca$^{2+}$-mediated pathways is not completely clear. However, the results of our 5′-deletion analysis indicate that PGE$_1$, 8-Br-cAMP, PMA, and okadaic acid all stimulate transcription through the same response element. Moreover, our results obtained using a yeast Gal4/CREB trans-reporting system indicate that PGE$_1$, 8-Br-cAMP, and PMA all activate CREB. In many cases, differential regulation of the α and β subunits of the Na,K-ATPase has been reported. Nevertheless, in this particular case, coordinate regulation of the human α and β subunit genes is quite possible, as an ATF/CRE site-GC box has also been localized in a similar manner in the human α$_1$ subunit promoter, although its functional properties have not been studied. Even when assuming that the α and β subunit genes are subject to coordinate regulation by PGE$_1$, cAMP, and PMA, the presence of an ATF/CRE site-GC box on both promoters does not necessarily indicate that PGE$_1$, cAMP, and PMA stimulate transcription of the α and β subunit genes to the same extent. However, even this latter case does not exclude the possibility of differential regulation of α and β subunit mRNA levels by PGE$_1$ and 8-Br-cAMP, as differential regulation may possibly also be the consequence of differences in control at the post-transcriptional level.

Very likely, this is a common theme in molecular biology, as promoters for a number of other genes ranging from hydroxymethylglutaryl-CoA reductase (56) to cyclin D$_3$ (57) to protein phosphatase 2Aα (18) have similar ATF/CRE site-GC boxes (50). However, our study is unique in demonstrating a common link to these regulatory elements and the cAMP and Ca$^{2+}$ signaling pathways. Further studies are in progress to determine the manner by which Sp1 and CREB interact to promote gene expression via these two signaling pathways.

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Identification of a Prostaglandin-responsive Element in the Na,K-ATPase β1 Promoter That Is Regulated by cAMP and Ca^{2+}: EVIDENCE FOR AN INTERACTIVE ROLE OF cAMP REGULATORY ELEMENT-BINDING PROTEIN AND Sp1

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