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Evidence for Post-transcriptional Regulation of Na, K-ATPase by Prostaglandin E₁

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Abstract

The stimulatory effect of PGE₁ on the activity of the Na, K-ATPase in MDCK cells is associated with an increase in the rate of transcription of the Na, K-ATPase β 1 subunit gene, and an increase in the rate of biosynthesis of the Na, K-ATPase [1]. In order to further define the molecular mechanisms, transient transfection and biosynthesis studies were conducted with Dibutyl cAMP resistant (DB^r) MDCK cells, defective in cAMP dependent protein kinase, and PGE₁ Independent (PGE₁ Ind) MDCK cells with elevated intracellular cAMP. Transient transfection studies with the human Na, K-ATPase β 1 promoter/luciferase construct, pH β 1-1141 Luc [2], showed that the stimulatory effect of PGE₁ and 8Br-cAMP on β 1 subunit gene transcription is retained in the DB^r and PGE₁ independent variants. However, the stimulatory effect of PGE₁ and 8Br-cAMP on Na, K-ATPase biosynthesis was lost in DB^r (unlike PGE₁ Ind) variants. These results can be explained by a defect in post-transcriptional regulation.

Keywords

prostaglandins; Na; K-ATPase; kidney; MDCK; cAMP; Protein Kinase C

Introduction

Prostaglandins are a family of biologically potent lipid acids produced following the sequential oxidation of Arachidonic Acid (AA) by Cyclooxygenase (Cox) and terminal prostaglandin synthase. Prostaglandins are produced at high levels in a number of tissues, affecting a broad range of processes, including blood pressure regulation, inflammation, as well as smooth muscle contraction [3]. The kidney is a major site of prostaglandin biosynthesis, where renal prostaglandins regulate renal blood flow, glomerular filtration, renal tubular sodium reabsorption, and ultimately blood pressure [4–6]. Sodium is reabsorbed by a number of different transport systems in renal tubule epithelial cells. Although the transport systems vary from one nephron segment to another, a number of transport systems which are involved in sodium reabsorption along the entire nephron are regulated by prostaglandins, including the Na, K-ATPase [7].

Of particular interest to this report are the effects of prostaglandins on the renal Na, K-ATPase. The Na, K-ATPase maintains transmembrane electrochemical gradients in all mammalian cells by through the transport of extracellular K⁺ in exchange for intracellular Na⁺ [8]. In the kidney, the electrochemical gradient established by the Na, K-ATPase creates the driving force for the reabsorption of amino acids, sugars and other solutes. The Na, K-ATPase is composed of an α subunit, responsible for the catalytic activity, and a β subunit, required for the integration of

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the Na, K-ATPase into the plasma membrane [9]. In addition, the β subunit has been found to be rate-limiting in the formation of α/β heterodimers in a number of cell types, including MDCK [10,11]. Thus changes in β subunit levels are sufficient to alter the level of the Na, K-ATPase in the plasma membrane, even when the Na, K-ATPase β subunit gene is regulated differentially from the α subunit gene by hormones and other effector molecules.

To study the mechanisms through which prostaglandins affect the renal Na, K-ATPase, we have conducted in vitro studies with Madin Darby Canine Kidney (MDCK) cell line, a model of renal distal tubule cells [12]. MDCK cells possess a polarized morphology in vitro, as well as the capacity for transepithelial Na^+ transport. Na^+ is transported into the cells by a Na^+/H^+ antiport system (localized on the apical membrane), and is transported out of the cells by the Na, K-ATPase (localized on the basolateral membrane) [13].

In our studies of the regulation of the Na, K-ATPase in MDCK cells by prostaglandins, we utilize a hormonally defined, serum-free medium [14–16]. PGE_1 , a growth supplement in this medium, stimulates transport via the Na, K-ATPase, as well as growth [16,17]. Our investigations with variant MDCK cells indicate that the stimulatory effects of PGE_1 on the Na, K-ATPase occur by means of signaling pathways which are distinct from those responsible for the growth stimulatory effect of PGE_1 [1,17]. Indeed PGE_1 independent variants (PGE_1 Ind) of MDCK cells retain the stimulatory effect of PGE_1 on the Na, K-ATPase, while having lost the stimulatory effect of PGE_1 on growth [1,18,19]. The PGE_1 Ind MDCK cells had elevated intracellular cAMP levels, which can be attributed to their decreased cAMP phosphodiesterase activity [18]. In contrast Dibutyl cAMP resistant (DB^r) MDCK cells retain the stimulatory effect of PGE_1 on growth, but have lost the stimulatory effect of PGE_1 on the Na, K-ATPase [17]. The DB^r MDCK cells are defective in type I cAMP dependent protein kinase.

In this report the PGE_1 Ind and DB^r variants have been studied further to determine the molecular sites that PGE_1 affects so to regulate the Na, K-ATPase in MDCK cells. Previously, we observed that PGE_1 increased Na, K-ATPase biosynthesis, as well as Na, K-ATPase mRNA levels (the $\beta 1$ subunit mRNA being primarily effected). Transient transfection studies conducted with a $\beta 1$ subunit promoter/luciferase construct (pH $\beta 1$ -1141 Luc) [20] showed a stimulatory effect of PGE_1 , which was mediated by cAMP, Ca^{2+} and Protein Kinase C (PKC). In this report, we conduct similar transient transfection studies and biosynthesis studies with DB^r and PGE_1 Ind MDCK cells in order to determine whether the effects of PGE_1 on Na, K-ATPase levels can be attributed primarily to regulation at the transcriptional level, or whether post-transcriptional regulation is also involved.

Materials and methods

Materials

Hormones, human transferrin, PGE_1 , and other chemicals were from Sigma. Dulbecco's modified Eagle's medium (DMEM), Nutrient Mixture F-12, soybean trypsin inhibitor, and lipofectamine were from Invitrogen Corp. (Carlsbad, CA). BioMax MS-2 film, and ^{35}S -methionine were from Perkin Elmer Life and Analytical Sciences (Boston, Mass). The Galacto-StarTM system was from Applied Biosystems (Bedford, MA). The Prism 4 program was obtained from GraphPad Software, Inc. (San Diego, CA). Affinity-purified rabbit polyclonal antibodies against the Na, K-ATPase α and β subunits were from Santa Cruz Biotechnology (Santa Cruz, Calif). Nitrocellulose membranes, Immun-Star AP Detection Kits, molecular weight markers, acrylamide, the Bio-Rad DC protein assay, and other chemicals for electrophoresis were from Bio-Rad (Hercules, CA). Autofluor was obtained from National Diagnostics (Atlanta, Georgia), and streptavidin sepharose beads were from GE Healthcare, Inc. (Piscataway, NJ). Reporter Lysis Buffer and the pSV β gal expression vector were from

Promega (Madison, WI). The human $\beta 1$ promoter/luciferase construct pH $\beta 1$ -1141 Luc (Dr. Jerry Lingrel, University of Cincinnati), was constructed by inserting the Na, K-ATPase $\beta 1$ subunit gene promoter into the expression vector pOLuc [21].

Cell Culture Methodology

The basal medium for MDCK cells was 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, 92 units/ml penicillin, and 200 μ g/ml streptomycin (DME/F12). DME/F12 was further supplemented with growth factors immediately prior to use [16]. Stock cultures of normal MDCK cells and DB^r 3 cells were grown in DME/F12 supplemented with 5 μ g/ml bovine insulin, 5 μ g/ml human transferrin, 5×10^{-12} M triiodothyronine (T_3), 5×10^{-8} M hydrocortisone, 25 ng/ml PGE₁, and 5×10^{-8} M selenium (Medium K-1) in a humidified 5% CO₂/95% air environment at 37°C. Stock cultures of PGE₁ Independent Clone I (Ind Cl I) were cultured in Medium K-1 lacking PGE₁. MDCK cells were routinely subcultured using 0.53 mM EDTA and 0.05% trypsin in Phosphate-Buffered Saline (PBS). After the cell detachment, trypsin action was inhibited using 0.1% soybean trypsin inhibitor in PBS [16].

Transient Transfection Studies

Normal and variant MDCK cells were plated (10^5 cells/35-mm dish) into culture dishes containing DME/F12 supplemented with 5 μ g/ml bovine insulin and 5 μ g/ml human transferrin. The next day the cultures were cotransfected (lipofectamine method) with 1 μ g pH $\beta 1$ -1141 Luc, and 0.2 μ g pSV β gal to standardize for transfection efficiency. Transfections were performed in antibiotic free DME/F12 supplemented with 5 μ g/ml insulin and 5 μ g/ml transferrin, using 4 dishes/condition. The next day, the medium was changed to DME/F12 supplemented with 5 μ g/ml insulin, 5 μ g/ml transferrin. After 2 hr (at 37°C, in a 5% CO₂/95% air humidified environment), appropriate effector molecules were added. Four hrs later, monolayers were solubilized in Reporter Lysis Buffer, and centrifuged (14,000 rpm; 1 min).

To measure luciferase activity, an aliquot of the cell lysate was placed in a microfuge tube containing 100 μ l luciferase assay buffer (20 mM Tricine, 1.07 mM MgCO₃·4Mg(OH)₂, 2.67 mM MgSO₄, 0.1 mM EDTA, 33.3 mM dithiothreitol, 270 μ M Coenzyme A, 470 μ M luciferin, and 530 μ M ATP). The light units emitted were counted in a Packard Tri-Carb 4530 scintillation counter with the coincidence circuit turned off. To determine β -galactosidase activity, aliquots of cell extracts were incubated at 23°C in a reaction buffer containing the Galacton-Star→ substrate. The emitted light was measured as described above.

The luciferase activity of each sample was normalized with respect to its β -galactosidase activity. In each condition, the normalized luciferase activity was determined by calculating the mean $\pm$ S.E. of quadruplicate determinations. The normalized value in each condition was compared fold-wise with the indicated control value from the same experimental culture set. The significance of each observed stimulatory (or inhibitory) effect was determined by means a one-way analysis of variance (ANOVA), and the Newman-Keuls multiple comparison test, using Prism 4 software. Differences were significant when $p < 0.05$.

Protein Synthesis Studies

Normal and variant MDCK cells were grown to confluence in Medium K-1 lacking PGE₁ (Control Medium). The cultures were maintained for 4 days either in Control Medium (-E), in Control Medium supplemented with 25 ng/ml PGE₁ (+E) or in Control Medium supplemented with 1 mM 8Br-cAMP and 0.5 mM isobutyl methylxanthine (IBMX) (+8Br). After 4 days MDCK monolayers were preincubated 20 minutes in methionine-free DME/F12 with the same supplements at 37°C in a 5% CO₂/95% air humidified environment, followed by a 1 hr incubation with [³⁵S]-methionine (0.125 μ Ci/ml) in methionine-free DME/F12 containing the

same factors. The monolayers were then solubilized in extraction buffer (0.1 M Tris [hydroxymethyl] aminomethane hydrochloride, pH 7.2/0.15 M NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate, 1.0% aprotinin, and 0.02% sodium azide), removed from the dishes with a rubber policeman, and transferred into microfuge tubes. The solubilized material was sonicated (4°C), microfuged (14,000 rpm; 5 min), and the protein content determined using the Bio Rad DC assay. The total protein content did not differ significantly in confluent cultures maintained under the conditions described above.

Immunoprecipitations were conducted as previously described by Taub et al. [22] with modifications. To reduce nonspecific binding, aliquots of cell extracts (equalized with respect to protein) were incubated with preimmune serum coupled to Protein G Sepharose in PTA (PBS (pH 7.2) containing 0.5% Tween 20, 0.05% SDS, 0.1% bovine serum albumin, and 0.02% sodium azide). The samples were then mixed by rotation for 2 hr (4°C) with a mouse monoclonal anti-Na, K-ATPase α 1 antibody (C464.6, Santa Cruz Biotech), or a mouse monoclonal anti-Na, K-ATPase β 1 antibody (C464.8, Santa Cruz Biotech) that were coupled to Protein G Sepharose in PTA. The beads were washed by centrifugation (3,000 rpm; 2x) in PTA, and heated in sample buffer (2% SDS/dithiothreitol; 100°C for 3 min.). Beads were removed by centrifugation (5 min; 14,000 rpm).

The immunoprecipitated samples (in sample buffer) were separated on 7.5% SDS Polyacrylamide gels [23]. Gels were soaked in Autofluor, an autoradiographic enhancer, and exposed to X-ray film (Kodak MR) at -70°C. The bands on X-ray film corresponding to the α and β subunits of the Na, K-ATPase were identified by their molecular weight, as well as by their co-migration with the α and β subunits of purified dog Na, K-ATPase. The intensities of the bands on the X-ray film were compared to control levels (-PGE₁) after scanning the images of the bands, using the Quantity One Program.

Results and Discussion

Effect of Prostaglandins and 8Br-cAMP on Transcription

In our previous transient transfection studies, our results indicated that PGE₁ and 8Br-cAMP were both stimulatory to transcription of the Na, K-ATPase β 1 subunit gene. Possibly, the defect in type I cAMP dependent protein kinase in DB^r 3 cells (a dibutyryl cAMP resistant clone) would result in the loss of the stimulatory effect of PGE₁ and 8Br-cAMP on transcription of the β 1 subunit gene. In order to determine whether such a stimulation of transcription was retained in DB^r 3 variant cells, transient transfection studies were conducted with the Na, K-ATPase β 1 subunit promoter/luciferase construct pH β 1-1141 Luc [2]. Parallel experiments were conducted with normal MDCK cells, as well as with a PGE₁ independent variant clone, Ind Cl I, which has lost the stimulatory effect of PGE₁ on growth.

The results of our studies (Figure 1A) indicate that 25 ng/ml PGE₁ and 1 mM 8Br-cAMP are stimulatory to transcription in DB^r 3 and PGE₁ Ind Cl I as well as normal MDCK cells. The stimulatory effects of PGE₁ and 8Br-cAMP in DB^r 3 cells (5.9 $\pm$ 1.5 fold and 7.6 $\pm$ 0.2 fold, respectively) were not reduced significantly in comparison with the stimulation of transcription observed in normal MDCK cells (5.1 $\pm$ 0.5 fold, PGE₁; 4.7 $\pm$ 0.8 fold, 8Br-cAMP), despite the decreased cAMP dependent protein kinase activity of these variant cells. Similarly, the magnitude of the PGE₁ and 8Br-cAMP stimulation obtained with PGE₁ Ind Cl I (4.9 $\pm$ 0.3 fold, PGE₁; 5.4 $\pm$ 0.4 fold, 8Br-cAMP) was not significantly reduced as compared with normal MDCK cells. Fig. 1B shows that a significant stimulatory effect of 10⁻⁹ M phorbol 12-myristate 13-acetate (TPA) was obtained with PGE₁ independent clone I (5.6 $\pm$ 1.7 fold) as well as normal MDCK cells (7.4 $\pm$ 1.1 fold), unlike the case with DB^r 3 cells (1.7 $\pm$ 0.5 fold).

The effects of prostaglandins were studied in greater detail. The dependence of the PGE₁ stimulation (Y) upon the PGE₁ concentration (X) was examined in normal MDCK, DB^r 3 and PGE₁ Ind Cl I (Figure 2). By means of nonlinear regression analysis, the results obtained with each cell type was fit to curve with the equation, $Y = V_{\max} (X)/(K_D + X)$, where $V_{\max}$ is the maximal stimulation, and K_D is the concentration of ligand (PGE₁) required to achieve half-maximal stimulation. With normal MDCK cells the K_D value was 7.9 ± 1.4 ng/ml PGE₁, and the $V_{\max}$ was a 9.5 ± 0.3 fold stimulation. Possibly, the defect in cAMP dependent protein kinase would cause DB^r 3 cells to be less responsive to PGE₁, especially at suboptimal concentrations. Indeed, the results with DB^r 3 cells were consistent with a higher K_D (15.0 ± 3.0 ng/ml PGE₁), without a change in $V_{\max}$. However the K_D value did not differ significantly from MDCK, given 95% confidence intervals. In PGE₁ Ind Cl I, the $V_{\max}$ value (12 ± 4 fold) was significantly higher than the MDCK value, without a change in K_D .

In addition, the stimulatory effects of different prostaglandins were also compared normal MDCK cells, and DB^r 3 cells (Figure 3). In MDCK cells 100 ng/ml PGE₁, PGE₂, PGA₁ and PGF₂ α all had equivalent stimulatory effects (≥ 4 fold). In contrast, in DB^r 3 cells only PGE₁, PGE₂ and 16,16 dimethyl PGE₂ showed responses of this magnitude. The spectrum of prostaglandin responsiveness observed with DB^r 3 cells at 100 ng/ml prostaglandin resembled the responsiveness previously observed with normal MDCK cells at a lower prostaglandin concentration (25 ng/ml) [24]. Thus these results are consistent with the hypothesis that DB^r 3 cells have a higher K_D values for prostaglandins.

Previously, we presented evidence indicating that PGE₁ stimulates $\beta 1$ subunit gene transcription through its interaction with 2 different classes of prostaglandin receptors, including Gq coupled EP1 receptors, which activate Phospholipase C, and Gs coupled EP2 receptors which activate adenylate cyclase [25,26]. The PGE₂ analog 16,16-dimethyl-PGE₂ has a 10 fold higher affinity for EP1 than EP2 receptors [27], and thus may only activate EP2 receptors in normal MDCK cells at high prostaglandin concentrations. The stimulatory effect of 16,16-dimethyl-PGE₂ in DB^r 3 cells is nonetheless very likely through EP1 receptors in this study, as signaling through EP1, which activate phospholipase C, is very likely unaltered. In contrast signaling events initiated through EP2 receptors (which activate adenylate cyclase) may require higher PGE₁ concentrations to elicit a maximal response in DB^r 3 cells, as a consequence of their defect in cAMP dependent protein kinase.

Effect of PGE₁ and 8Br-cAMP on the Biosynthesis of the Na, K-ATPase

Previously, we showed that the stimulatory effect of PGE₁ and 8Br-cAMP on Na, K-ATPase $\beta 1$ subunit gene transcription is associated with an increase in the rate of biosynthesis of the Na, K-ATPase in MDCK cells [26], which is presumably responsible for the increased Na, K-ATPase activity observed under these conditions. Possibly, PGE₁ and 8Br-cAMP have similar effects on Na, K-ATPase biosynthesis in PGE₁ Ind Cl I, and DB^r 3 cells. Indeed both PGE₁ and 8Br-cAMP stimulate Na, K-ATPase $\beta 1$ subunit gene transcription in PGE₁ Ind Cl I and DB^r 3 cells. However, DB^r 3 cells do not respond to either PGE₁ or 8Br-cAMP with an increase in Na, K-ATPase activity (unlike PGE₁ independent MDCK cells). Alternatively, the alteration in DB^r 3 cells does indeed affect the regulation of Na, K-ATPase biosynthesis.

In order to evaluate this possibility, metabolic labeling studies were conducted with DB^r 3 cells, in addition to PGE₁ Ind Cl I, and normal MDCK cells. The cultures were grown to confluence either in defined Medium K-1 lacking PGE₁, or in Medium K-1 with PGE₁. Monolayers were then maintained 4 additional days in Medium K-1 further incubated with either 25 ng/ml PGE₁, 1 mM 8Br-cAMP, or with no further supplement. At the end of the 4 day incubation, the monolayers were labeled with ³⁵S-methionine. The Na, K-ATPase was immunoprecipitated from extracts of ³⁵S-labeled cells, and separated by SDS-PAGE. Radioactive Na, K-ATPase was detected by autoradiography.

In normal MDCK cell cultures treated with PGE₁, the level of both the α subunit (~100 Kd) increased 6.2 fold (Fig. 4A). The level of glycosylated β subunit (approximately 55 Kd) increased 3.6 fold and the level of unglycosylated β subunit (~33 kd) increased 3.0 fold in the presence of PGE₁ (Fig. 4B). Similarly, the level of ³⁵S labeled Na, K-ATPase α and β subunits increased by 3.6 fold in the presence of 8Br-cAMP (Figure 4A). The level of glycosylated β subunit increased 5.3 fold, and unglycosylated β subunit increased by 2.9 fold, respectively (Figure 4B). Total protein synthesis was not similarly affected (Figure 4C).

Fig. 4 also shows the effect of PGE₁ and 8Br-cAMP on the biosynthesis of the Na, K-ATPase in DB^r 3 cells and PGE₁ Ind Cl I. In DB^r 3 cells, PGE₁ and 8Br-cAMP did not stimulate the biosynthesis of the Na, K-ATPase α and β subunits. In contrast, stimulatory effects of PGE₁ and 8Br-cAMP were retained in PGE₁ independent clone I, indicating that the stimulatory effects of PGE₁ and 8Br-cAMP could not simply be explained by indirect effects on cell viability.

Discussion

The Na, K-ATPase is subject to regulation at the transcriptional level, in response to a number of hormones and other effector molecules, including mineralocorticoids, glucocorticoids, and thyroid hormone [20]. However evidence for post-transcriptional regulation of the Na, K-ATPase has been obtained. For example in chicken skeletal muscle cells, of veratridine causes an increase in the rate of transcription of the Na, K-ATPase β 1 subunit gene, and a transient 3 fold increase in the level of the β 1 subunit mRNA [28]. Following the transient increase in β subunit mRNA, β mRNA levels fall abruptly, reflecting a decrease in β mRNA stability. The stability of mRNA molecules can be affected by the binding of trans-acting binding proteins to the 3' untranslated region [29–31]. Indeed the Na, K-ATPase β 1 subunit mRNA is AU-rich, and contains three AUUA motifs (also called adenylate- and uridylylate-rich elements, or AREs) [32]. Such AREs can be recognized by a number of trans-acting factors, such as AUF1 and HUR [31], that may affect either mRNA stability or translational efficiency. Because protein kinase A and protein kinase C are both known to regulate mRNA stability, their possible effects on trans-acting factors which bind to AREs has been examined. In the case of the lactate dehydrogenase-A subunit mRNA the protein kinase A-regulated instability has been shown to be dependent upon the presence of AREs in the 3'-UTR, and thus implicating the involvement of specific trans-acting factors [33].

In this study we have presented evidence for post-transcriptional regulation of the Na, K-ATPase β 1 subunit gene by protein kinase A. In dibutyryl cAMP resistant MDCK cells, transcriptional regulation of the Na, K-ATPase β 1 subunit gene by PGE₁ and 8Br-cAMP remains intact, despite the defect in type I cAMP dependent protein kinase. However the stimulatory effects of PGE₁ and 8Br-cAMP on the biosynthesis of the Na, K-ATPase are no longer observed in these variant cells. Presumably, the defect in protein kinase A does prevent the stimulatory effect of PGE₁ on transcription, because of the remaining kinase activity (possibly type II cAMP dependent protein kinase) is sufficient to elicit a transcriptional effect, in combination with PKC. However, the stimulatory effect of PGE₁ on Na, K-ATPase biosynthesis is lost nevertheless in the dibutyryl cAMP resistant variants. This latter observation suggests that another regulatory event is also required in order to obtain the PGE₁ mediated increase in Na, K-ATPase biosynthesis in normal MDCK cells, and that this regulatory event is impaired by the defect in protein kinase A in DB^r 3 cells. Further studies are in progress in order to determine whether such cAMP mediated regulation affects either Na, K-ATPase β 1 subunit mRNA stability and/or the translational efficiency of Na, K-ATPase β 1 subunit mRNA.

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Figure 1.

Regulation by agents that affect cAMP and calcium metabolism. MDCK cells, DBr 3 cells, and PGE₁ Independent Cl I were transiently transfected with pH β 1-1141 Luc and pSV β gal. A) The effect of 25 ng/ml PGE₁ and 1 mM 8Br-cAMP on luciferase gene expression was determined following a 4 hr incubation. B) The effect of 10⁻⁹ M phorbol 12-myristate 13-acetate (TPA) and 1 mM 8Br-cAMP on luciferase gene expression was determined following a 4 hr incubation.

Figure 2.

Effect of PGE₁ Concentration on β 1 Subunit Gene Transcription. Normal MDCK cells, DBr 3 cells and PGE1 Independent CI I were transiently transfected with pH β 1-1141 Luc, as well as pSV β gal, a control for transfection efficiency. The effect of PGE₁ on luciferase gene expression was determined as indicated at PGE₁ concentrations ranging from 2.5–250 ng/ml following a 4 hr treatment. The best fit to the data to the equation $Y = V_{max} X / (X + K_D)$ was obtained by nonlinear regression analysis, using the Prism 4 program.

Figure 3. Effect of Different Prostaglandins on Transcription. MDCK cells were transiently transfected with pH β 1-1141 Luc, as well as pSV β gal. The effects of different prostaglandins on luciferase gene expression was determined following a 4 hr incubation, using a prostaglandin concentration of 100 ng/ml.

Figure 4.

Effect of PGE₁ and 8Br-cAMP on Na, K-ATPase Biosynthesis. MDCK monolayers were grown to confluence without PGE₁, and then were maintained for 4 days either with i) no further additive, ii) 25 ng/ml PGE₁ (+E), or ii) 1 mM 8Br-cAMP + 0.5 mM IBMX. After radiolabeling with ³⁵S-methionine for 2 hours, the Na, K-ATPase was immunoprecipitated. A) Immunoprecipitates of the Na, K-ATPase and B) samples of total ³⁵S-labeled protein were separated on SDS gels, and subjected to autoradiography, as described in Materials and Methods. Two bands labeled β' represent glycosylated β subunits, whereas the band labeled β represents the unglycosylated β subunit. The relative intensity of the α and β subunit bands in comparison with the -E condition was determined by scanning densitometry, with the 2 β' values averaged. The values for the relative intensity of labeling of total protein relative to the -E condition (determined by scanning densitometry) was for MDCK cells, 0.6 (+E), 0.4 (8Br-cAMP), and 0.8 (K1); for DB'3 cells, 1.3 (+E), 0.8 (8Br-cAMP), and 1.0 (K1), and for Ind Cl I cells, 0.9 (+E), 1.1 (8Br-cAMP) and 0.4 (K1).