Coordinate control of prostaglandin E₂ synthesis and uptake by hyperosmolarity in renal medullary interstitial cells

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Pucci, Michael L., Shinichi Endo, Teruhisa Nomura, Run Lu, Cho Khine, Brenda S. Chan, Yi Bao, and Victor L. Schuster. Coordinate control of prostaglandin E₂ synthesis and uptake by hyperosmolarity in renal medullary interstitial cells. Am J Physiol Renal Physiol 290: F641–F649, 2006. First published November 1, 2005; doi:10.1152/ajprenal.00426.2004.—During water deprivation, prostaglandin E₂ (PGE₂), formed by renal medullary interstitial cells (RMICs), feedback inhibits the actions of antidiuretic hormone. Intersitial PGE₂ concentrations represent the net of both PGE₂ synthesis by cyclooxygenase (COX) and PGE₂ uptake by carriers such as PGT. We used cultured RMICs to examine the effects of hyperosmolarity on both PGE₂ synthesis and PG uptake in the same RMIC. RMICs expressed endogenous PGT as assessed by mRNA and immunoblotting. RMICs rapidly took up [³H]PGE₂ to a level 5- to 10-fold above background and with a characteristic time-dependent “overshoot.” Inhibitory constants (Kᵢ) for various PGs and PGT inhibitors were similar between RMICs and the cloned rat PGT. Increasing extracellular hyperosmolarity to the range of 335–485 mosM increased the net release of PGE₂ by RMICs, an effect that was concentration dependent, maximal by 24 h, reversible, and associated with increased expression of COX-2. Over the same time period, there was decreased cell-surface activity of PGT due to internalization of the transporter. With continued exposure to hyperosmolarity over 7–10 days, PGE₂ release remained elevated, COX-2 returned to baseline, and PGT-mediated uptake became markedly reduced. Our findings suggest that hyperosmolarity induces coordinated changes in COX-2-mediated PGE₂ synthesis and PGT-mediated PGE₂ uptake in RMICs.

PRODUCTION OF CONCENTRATED urine by the mammalian kidney is dependent on two interrelated antidiuretic hormone (ADH)-dependent processes: 1) the establishment of a hyperosmolar environment in the renal medullary interstitium by the countercurrent multiplier mechanism and 2) a selective increase in collecting duct water permeability (18). Prostaglandins (PGs), specifically PGE₂, act as pharmacological antagonists of ADH on the medullary thick ascending limb of Henle (mTALH) and the collecting duct and, therefore, induce water diuresis (34).

RMICs rapidly took up [³H]PGE₂ to a level 5- to 10-fold above background and with a characteristic time-dependent “overshoot.” Inhibitory constants (Kᵢ) for various PGs and PGT inhibitors were similar between RMICs and the cloned rat PGT. Increasing extracellular hyperosmolarity to the range of 335–485 mosM increased the net release of PGE₂ by RMICs, an effect that was concentration dependent, maximal by 24 h, reversible, and associated with increased expression of COX-2. Over the same time period, there was decreased cell-surface activity of PGT due to internalization of the transporter. With continued exposure to hyperosmolarity over 7–10 days, PGE₂ release remained elevated, COX-2 returned to baseline, and PGT-mediated uptake became markedly reduced. Our findings suggest that hyperosmolarity induces coordinated changes in COX-2-mediated PGE₂ synthesis and PGT-mediated PGE₂ uptake in RMICs.

For PGs to modulate cellular events over short temporal and spacial domains, there must be local and rapid signal termination. Although some PGs, such as PGI₂, are inherently structurally unstable and inactivate spontaneously, PGE₂ and other PGs are very stable in whole blood or plasma (31). For these latter PGs, termination of signaling involves the two-step process of carrier-mediated uptake followed by cytoplasmic oxidation (31).

The uptake step in PG signal termination is mediated by the PG carrier PGT (22, 24, 28). PGT is widely expressed in situ in cell types that synthesize and release PGs, including endothelial cells, platelets, renal collecting duct principal cells, and RMICs (1, 3, 35). PGT transports PGE₂, PGF₂α, and PGD₂ at physiological affinities, i.e., the Kᵢ's are 50–100 nM range (22, 24, 28). PGT is a bidirectional lactate/PG exchanger (2, 9). Because cells of the renal medulla engage in substantial glycolysis and generate lactate (12), PGT is energetically poised to remove PGs from the renal medullary interstitium.

The present studies were designed to 1) develop cultured RMICs as a model system for studying the regulation of PGT and 2) examine the short- and long-term regulation of PGT, PG release, and cyclooxygenase (COX)-2 enzyme regulation in response to hyperosmolar media. We find that, on exposure to hyperosmolar media, COX-2 expression increases in the first 24 h but returns to control levels by 7–10 days. There is a sustained elevation of PGE₂ release over this extended time period that is accompanied by a similarly sustained reduction of PGT function at the plasma membrane.

MATERIALS AND METHODS

Materials. Unless otherwise noted, all chemicals of analytic grade or better were obtained from Sigma (St. Louis, MO). Tracer-PGE₂ ([³H]PGE₂) was obtained from DuPont New England Nuclear (Boston, MA). Primary cultures of rat RMICs were obtained courtesy of Dr. E. Nord (SUNY at Stony Brook, NY) at passage 132.

Cell culture. HeLa cells were grown on 35-mm dishes, infected with vaccinia vTF7-3, and transfected with a functional rat PGT cDNA as described (22). RMICs were maintained in humidified incubators with 5% CO₂-air at 37°C in the RMIC media: DMEM containing 10% fetal bovine serum, 100 U/ml each of penicillin and streptomycin, and 0.28 U/ml of bovine insulin. Unless otherwise noted, RMICs were grown to confluence as monolayers on 35-mm dishes for 2 days. At that point, the medium was replaced by RMIC media without or with added NaCl (25, 50, or 100 mM, equivalent to 50, 100, or 200 mosmol/kgH₂O) or urea (50, 100, or 200 mM, equivalent to 50, 100, or 200 mosmol/kgH₂O). Experiments were performed between 2 h and 10 days later.

Northern blotting and immunoblotting. Total RNA was extracted from RMICs and whole rat kidney as described previously (22) using guanidinium acid phenol extraction (11). A rat PGT RNA probe was generated using a 3ʹ-truncated PGT cDNA in the vector pGEM3Z; this was linearized with Nco1, and an anti-sense digoxigenin-labeled cRNA probe was generated with SP6 RNA polymerase (22). RNA

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was separated by glyoxal agarose gel electrophoresis, transferred to Hybond N, hybridized with the digoxigenin-labeled probe, and washed twice at 0.1 × SSC, 65°C, and visualized by chemiluminescence autoradiography (Amersham), 2-h exposure. The degree of lane loading was established by methylene blue staining and by probing for GAPDH.

For immunoblotting, cell monolayers were rinsed twice with ice-cold balanced salt solution (BSS) followed by ice-cold lysis buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 1 mM PMSF, 1 μg/μl leupeptin, 1 μg/μl pepstatin). Cells were incubated in lysis buffer for 20 min at 4°C. Lysates were collected by scraping and were centrifuged at 15,000 g for 5 min at 4°C. After protein concentration of the supernatant was measured as above, cell lysates (30 μg/lane) were subjected to SDS-PAGE (10% polyacrylamide). Proteins were separated by SDS-PAGE under reducing conditions (2 mM β-mercaptoethanol added to loading buffer) for immunoblot of COX-1 and COX-2 and under nonreducing conditions for immunoblot of PGT (3). Proteins were transferred to nitrocellulose membranes (Optitran, Schleicher & Schuell, Keene, NH) by electroporation. Blots were blocked (5% nonfat dry milk, 1% Tween 20 in PBS) for 1 h, then immunostained for 2 h with the following primary antibodies: anti-PGT [undiluted hybridoma cell culture media, anti-rat mouse monoclonal (3)], anti-COX-2 (1:1,000, anti-sheep goat polyclonal, Cayman Chemical, Ann Arbor, MI), or anti-COX-1 (1:1,000, anti-sheep mouse monoclonal, Cayman Chemical). After three rinses with PBS containing 1% Tween 20 (PBS/Tween), blots were incubated with appropriate horseradish peroxidase (HRP)-coupled secondary antibodies (1:5,000) in PBS/Tween, after which protein bands were detected by chemiluminescence (New England Nuclear). For quantification, blots were stained with Coomassie Blue and scanned for total protein per lane.

**PGE2 influx measurements.** Cell monolayers were rinsed twice with BSS (135 mM NaCl, 5 mM KCl, 13 mM Na-HEPES, 2.5 mM CaCl2, 1.2 mM MgCl2, 0.8 mM MgSO4, 25 mM glucose). PG influx was initiated by addition of BSS containing unlabeled PGE2 (1 μM). Release measurements were performed in the presence and absence of excess unlabeled PGE2 (1 μM) or brom cresol green (BCG; 10 μM). PGE2 release measurements. Cell monolayers were rinsed twice with BSS followed by addition of BSS without or with endothelin-1 (ET-1; 10 nM; Peptide International), calcium ionophore (A23187, 10 μM), or arachidonic acid (AA; 10 μM) for 5 min. Release assays were performed at 37°C. Assays were terminated by collection of an aliquot of assay solution. Aliquots were stored at −70°C until measurement of PGE2 concentration. Cells were lysed in 5% SDS and an aliquot was taken for protein measurement, as above. PGE2 concentrations in assay samples were measured by enzyme immunoassay (Cayman Chemical). Release values were calculated as the mean of duplicate monolayers and expressed as nanograms per milliliters per milligram of protein per min. In some experiments, RMICs were incubated for 15 min before and during PGE2 release measurements without or with a nonselective COX inhibitor [10 μM indomethacin (Indo)] or a selective COX-2 inhibitor (5 μM NS-398; Calbiochem, San Diego, CA).

**Immunocytochemistry.** RMICs grown on glass coverslips were blocked in 5–10% goat serum, incubated overnight at 4°C with mouse monoclonal antibody no. 20 as straight hybridoma supernatant (3), washed, and incubated for 1 h in FITC-coupled goat anti-mouse IgG at 1:2,000 (Alexa Fluor 488, Molecular Probes, Invitrogen, Eugene, OR). Negative controls consisted of omission of the primary antibody.
Photomicroscopy was performed using a Bio-Rad Laser Confocal Microscope.

Lactate concentration. Cell monolayers were grown overnight in media with and without 100 mosmol/kgH2O added NaCl, after which the medium was collected and assayed for lactate concentration using a commercially available kit (Sigma) that uses a colorimetric assay based on the enzymatic conversion of lactic acid to pyruvate and hydrogen peroxide by lactate oxidase. Due to the long incubation, it was assumed that lactate was in equilibrium between the intracellular and extracellular compartments and, therefore, extracellular [lactate] would be proportional to intracellular [lactate].

Statistical analysis. Values represent means ± SE. Statistical analysis was performed by Student’s t-test or by ANOVA followed by Newman-Kuels modified t-test as a post hoc analysis. The null hypothesis was rejected at P < 0.05.

RESULTS

Endogenous PGT expression in cultured RMICs. Rat RMICs were probed for expression of PGT mRNA and protein by Northern blotting and immunoblotting, respectively. Figure 1 shows that a single ~4.4-kb RNA band from cultured rat RMICs hybridized with a rat PGT antisense RNA probe (left). This mRNA band comigrated with a PGT band from whole rat kidney RNA (right); both were in accord with previously published results on rat PGT RNA from our laboratory (22). The additional band detected in whole rat kidney may derive from PGT expressed outside of RMICs by glomeruli, endothelia, and/or collecting ducts (3).

Similarly, as shown in Fig. 2, RMIC whole cell lysates probed with an anti-rat PGT monoclonal antibody (3) revealed a single immunoreactive band at ~65 kDa. This band is similar in size to that of PGT from rat kidney as reported previously (3) and is also similar in size to rat liver PGT (Fig. 2, right). The slight difference in migration between RMIC PGT and liver PGT is similar to variations we observed among various tissues or cell types (Bao Y and Schuster VL, unpublished observations). The basis for this variation is currently unknown but presumably is due to factors such as alternative RNA splicing and/or differential glycosylation.

To determine whether PGT protein in cultured RMICs is expressed functionally at the plasma membrane, we examined the timed uptake of radioactive PGs from the medium. Figure 3 demonstrates that [3H]PGE2 undergoes rapid, time-dependent uptake by cultured RMICs. In contrast, control HeLa cells, which lack PGT expression, exhibited very low [3H]PGE2 uptake. The “overshoot” pattern seen here with PGE2 uptake by cultured RMICs is characteristic of PGT-mediated PG transport and is probably due to the time-dependent dissipation of an outwardly directed lactate gradient during the uptake assay (9, 10).

The affinities of the RMIC uptake step for several prostanoids are compared with those of the cloned, recombinant rat PGT in Fig. 4 (21, 22, 32). Figure 4A shows the uptake of [3H]PGE2 in RMICs and in HeLa cells transiently transfected with recombinant rat PGT (“HeLa-PGT”) and the competition for the uptake step by unlabeled PGE2 (squares) and unlabeled TxB2 (circles) in HeLa cells expressing rat PGT (HeLa-PGT) and in RMICs. B: inhibition of [3H]PGE2 uptake by unlabeled PGE2α, in HeLa cells expressing rat PGT (HeLa-PGT) and in RMICs. C: inhibition of [3H]PGE2 uptake by unlabeled U46619 in HeLa cells expressing rat PGT (HeLa-PGT) and in RMICs.

Fig. 4. A: inhibition of [3H]PGE2 uptake by unlabeled PGE2 (squares) and unlabeled TxB2 (circles) in HeLa cells expressing rat PGT (HeLa-PGT) and in RMICs. B: inhibition of [3H]PGE2 uptake by unlabeled PGE2α, in HeLa cells expressing rat PGT (HeLa-PGT) and in RMICs. C: inhibition of [3H]PGE2 uptake by unlabeled U46619 in HeLa cells expressing rat PGT (HeLa-PGT) and in RMICs.
for this uptake by unlabeled PGE\(_2\) or thromboxane B\(_2\) (TxB\(_2\)) as a function of their concentrations in the uptake solution. Although the dose-response curves are offset from each other, unlabeled PGE\(_2\) and unlabeled TxB\(_2\) result in similar competition curves in RMICs and HeLa-PGT cells. Double-reciprocal plots (not shown) indicated that the inhibitor constants (K\(_i\), nM) for PGE\(_2\) were 178 and 176 nM in RMIC and HeLa-PGT cells, respectively, and for TxB\(_2\) were 709 and 682 nM in RMIC and HeLa-PGT cells, respectively (P = NS for all RMIC vs. HeLa-PGT comparisons). Similarly, Fig. 4B shows that PGE\(_2\) had a similar affinity for the transporter in RMICs and in HeLa-PGT cells; the relevant K\(_i\) values were 462 nM for RMICs and 362 nM for HeLa-PGT cells. Figure 4C demonstrates similar results for the prostacyclin agonist U46619, for which the K\(_i\) values were 823 nM for RMICs and 1,059 nM for HeLa-PGT cells (P = NS for all RMIC vs. HeLa-PGT comparisons).

We extended this analysis by examining the inhibition of tracer PGE\(_2\) uptake by a single fixed dose of each of several other eicosanoids that exhibit lower affinities for rat PGT (21, 32). As shown in Table 1, PGE\(_2\) isopropylester, 15-keto PGE\(_2\), and 13,14 dihydro 15-keto PGE\(_2\) showed comparable inhibition, or lack thereof, in RMICs compared with HeLa-PGT cells.

To complete the characterization of endogenous PGT in RMICs, we compared the affinities of several organic anion transport inhibitors that block rat PGT. Figure 5 shows the dose-dependent inhibition of \([\text{H}]\)PGE\(_2\) uptake by BCG (22), DIDS (10), and indocyanine green (ICG) (22). As with competition by the unlabeled eicosanoids, these organic anions blocked PG uptake in RMICs and HeLa-PGT over comparable dose-response ranges. Double-reciprocal plots indicated that the relevant K\(_i\) values were as follows. For BCG: 8.7 \pm 1.0 \mu M for RMICs and 12.3 \pm 1.2 \mu M for HeLa-PGT cells; for DIDS: 32.2 \pm 1.2 \mu M for RMICs and 42 \pm 2.5 \mu M for HeLa-PGT cells; and for ICG: 2.7 \pm 0.3 \mu M for RMICs and 1.6 \mu M for HeLa-PGT cells (P = NS for all RMIC vs. HeLa-PGT comparisons).

### Table 1. Competition of PGE\(_2\) uptake by other eicosanoids

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<thead>
<tr>
<th>PG Competitor</th>
<th>RMIC</th>
<th>HeLa-PGT</th>
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<tbody>
<tr>
<td>None</td>
<td>100(\pm)2.5</td>
<td>100(\pm)4.6</td>
</tr>
<tr>
<td>PGE(_2) isopropylester</td>
<td>59(\pm)6.1</td>
<td>62(\pm)5.4</td>
</tr>
<tr>
<td>15-keto PGE(_2)</td>
<td>88(\pm)0.3</td>
<td>67(\pm)0.4</td>
</tr>
<tr>
<td>13,14 dihydro 15-keto PGE(_2)</td>
<td>122(\pm)1.7</td>
<td>105(\pm)1.9</td>
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Values are means \(\pm\) SE. HeLa cells expressed rat PGT as per text. P = not significant for renal medullary interstitial cells (RMIC) vs. HeLa for each competitor.

NaCl. A similar, albeit less intense, effect on PGE\(_2\) uptake was elicited by urea (Fig. 7).

As shown in Fig. 8A, PGT function began to decrease rapidly after exposure to increased NaCl. The effect was maximal after 24 h and persisted for at least 120 h. The NaCl-induced decrease in PGT function was reversible on returning the cells to isosmolar media (Fig. 8B), although the return of function was somewhat slower than the previous decline.

One possible explanation for the change in tracer PG uptake was that the driving force for PG uptake by PGT, i.e., the cell-to-medium lactate concentration gradient (9), was altered by hyperosmolarity. However, cellular lactate concentrations at the start of the timed uptake assay were not different in control

### Fig. 5. Inhibition of \([\text{H}]\)PGE\(_2\) uptake by the organic anion transport inhibitors brom cresol green (BCG), 4,4’-diisothiocyanatodihydrostilbene-2,2’-disulfonate (DIDS), and indocyanine green (ICG) in HeLa cells expressing rat PGT (HeLa-PGT) and in RMICs.

### Fig. 6. Effect of added NaCl on PGT function. Confluent cultures of RMICs were grown for 24 h in media containing 0, 50, 100, or 200 mosmol/kgH\(_2\)O excess NaCl. Uptake of \([\text{H}]\)PGE\(_2\) over 10 min was measured. Values are expressed as fmol/mg protein/nM PGE\(_2\). Data points are means \(\pm\) SE of 6 experiments done in duplicate. *P < 0.05.
cells and cells incubated with high salt (32.6 ± 6.2 vs. 36.8 ± 5.9 mM, for cells grown in media without or with 100 mosmol/kgH₂O additional NaCl, respectively).

We examined whether the change in PGT transport was associated with a parallel change in PGT cell-surface protein expression. Figure 9A indicates that in control RMICs, PGT was distributed primarily in punctate cytoplasmic vesicles. Despite the clear presence of PGT at the plasma membrane by uptake assays, we were unable to adequately quantify cell-surface PGT using biotinylation approaches, suggesting that most of the PGT resides within cytoplasmic vesicles. Importantly, exposure of cultured RMICs to hyperosmolar medium for 24 h caused a dramatic internalization of PGT to an apparently nuclear distribution (Fig. 9A). Immunoblotting revealed that this redistribution was not accompanied by a change in total cellular PGT protein expression (Fig. 9B).

Effect of increased osmolarity on net PGE₂ release by RMICs. To determine whether exposure of RMICs to increased osmolarity affected PG synthesis and release, we measured net PGE₂ release as stimulated by ET-1, the calcium ionophore A23187, or AA. As expected, under isosmolar conditions, all three agonists greatly stimulated net PGE₂ release from RMICs (in units of ng·mg protein⁻¹·min⁻¹·nM [³H]PGE₂⁻¹). Data points are means ± SE of 4 experiments done in duplicate. *P < 0.05.

As shown in Fig. 10, overnight incubation in media containing 50, 100, or 200 mosmol/kgH₂O excess NaCl further increased net PGE₂ release in response to these agonists. The increases were greater as osmolality increased. The induction of ET-1- and A23187-stimulated net release was comparable at each level of hyperosmolality, whereas the induction of AA-stimulated net PGE₂ release was less at the highest osmolality. The time course shown in Fig. 11 illustrates that, after exposure of RMICs to hyperosmolar media, A23187-stimulated net PGE₂ release increased rapidly in a fashion similar to the osmolarity-induced decrease in PGT function.

To determine the contribution of COX-2 to the synthesis of PGs by RMICs, we compared net PGE₂ release in the absence and presence of indomethacin or NS-398. As shown in Table 2, both COX inhibitors greatly decreased basal and A23187-induced net PGE₂ release from RMICs grown in isosmolar and hyperosmolar media. The proportional effect of NS-398 was similar in RMICs grown in isosmolar and hyperosmolar media. Thus, as reported by others (14–16, 25, 39), PGE₂ synthesis in RMICs is mediated primarily by COX-2 under both iso- and hyperosmotic conditions.

We also examined the effects of hyperosmolarity on COX-2 protein expression using immunoblot analysis. As illustrated in

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Fig. 7. Effect of added urea on PGT function. Confluent cultures of RMIC were grown for 24 h in media containing 0, 50, 100, or 200 mosmol/kgH₂O added urea. Uptake of [³H]PGE₂ over 10 min was measured. Values are expressed as fmol·mg protein⁻¹·nM [³H]PGE₂⁻¹. Data points are means ± SE of 6 experiments done in duplicate.

Fig. 8. A: time course of the effect of added NaCl on PGT function in RMICs. Cultured cells were grown for growing times in media containing 100 mosmol/kgH₂O excess NaCl. Uptake of [³H]PGE₂ over 10 min was measured. Values are expressed as fmol·mg protein⁻¹·nM [³H]PGE₂⁻¹. Data points are means ± SE of 4 experiments done in duplicate. B: time course of the effect of removal of excess NaCl on PGT function. Cells grown for >48 h in media with 100 mosmol/kgH₂O excess NaCl were switched to isosmolar media for varying times. Uptake of [³H]PGE₂ over 10 min was measured. Values are expressed as fmol·mg protein⁻¹·nM [³H]PGE₂⁻¹. Data points are means ± SE of 4 experiments done in duplicate.
Fig. 12. A and C, COX-2 increased approximately threefold after 24-h exposure to 100 and 200 mosmol/kgH2O added NaCl. A concentration-dependent increase in COX expression between 100 and 200 mosmol/kgH2O NaCl was not discernible.

Fig. 9. A: shift of localization of PGT. RMICs were immunolabeled with a monoclonal antibody to rat PGT after growth for 24 h in control isosmolar medium (left) or medium with 100 mosmol/kgH2O added NaCl. Hyperosmolarity induced a shift in PGT expression from a primarily punctate cytoplasmic distribution to a primarily nuclear distribution. B: immunoblot illustrating the effect of added NaCl on expression of immunoreactive PGT. RMIC were grown for 24 h in media with or without 100 mosmol/kgH2O excess NaCl. Cell lysates were prepared and proteins were separated by SDS-PAGE. Immunoblots prepared from the gels were probed with anti-PGT monoclonal antibody. Blot represents data from 3 separate experiments.

Long-term effects of increased osmolarity. Whereas most studies on hyperosmolarity focus on a short experimental time frame, water deprivation in nature can be sustained over time, e.g., during drought. To more closely mimic these events, we grew RMICs in media without or with 100 mosmol/kgH2O added NaCl for 7–10 days. The cells grew well with no apparent differences in doubling rate, viability, or subculturing efficiency compared with cultures grown in isosmolar media.

Fig. 10. Effect of added NaCl on endothelin-1 (ET-1), calcium ionophore (A23187), or arachidonic acid (AA)-induced PGE2 release from RMICs. Cells were grown without or with 50, 100, or 200 mosmol/kgH2O added NaCl for 24 h. RMICs were exposed to agonists for 5 min after which extracellular buffer was collected and assayed for PGE2 by EIA. Values are expressed as the fold-increase in PGE2 release of cells incubated with added NaCl compared with cells not exposed to added NaCl (control). Data points are means ± SE of 6 experiments done in duplicate. All increases over control have P values <0.05.

Fig. 11. Time course of the effect of added NaCl on PGE2 release by RMICs. Confluent cultures were grown for varying times in media containing 100 mosmol/kgH2O excess NaCl. Release of PGE2 into the extracellular buffer for 5 min after stimulation with calcium ionophore (A23187, 10 μM) was determined by EIA. Values are ng·mg protein−1·min−1. Data points are means ± SE of 4 experiments done in duplicate.
Table 2. Effects of COX inhibitors on PGE2 release from RMICs incubated for 24 h with or without 100 mosmol/kgH2O added NaCl (final = 385 mosmol/kgH2O)

<table>
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<tr>
<th></th>
<th>No added NaCl</th>
<th>100 mosmol/kgH2O added NaCl</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>A23187</td>
</tr>
<tr>
<td>Control</td>
<td>65±5</td>
<td>221±5*</td>
</tr>
<tr>
<td>Indo</td>
<td>2.2±2</td>
<td>4±4</td>
</tr>
<tr>
<td>NS-398</td>
<td>2.5±2</td>
<td>6±4</td>
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Values represent means ± SE for 4 experiments. *P < 0.05 relative to control of the same group. †P < 0.05 relative to the other group. COX, cyclooxygenase; Indo, indomethacin.

As illustrated in Fig. 12, B and C, COX-2 immunoreactivity did not remain elevated in lysates from cells grown in hyperosmolar media for 7–10 days. In separate studies, we found that COX-1 protein was also not elevated at these late time points (data not shown).

After 7–10 days in hyperosmolar media, basal and AA-stimulated net PGE2 release from RMICs were no longer higher than control (basal: 0.83 ± 0.10 vs. 1.04 ± 0.16: AA-stimulated: 44.6 ± 2.5 vs. 45.1 ± 2.3 ng·ml⁻¹·mg protein⁻¹, isosmolar vs. hyperosmolar, respectively). On the other hand, both ET-1- and A23187-stimulated net PGE2 release remained elevated (~6-fold over control; Fig. 13).

Because this increase in net agonist-induced PGE2 release occurred in the face of a return of COX-2 levels to control values, we examined PGT expression. As shown in Fig. 14, PGT function remained markedly suppressed in RMICs grown for 7–10 days in hyperosmolar media.

DISCUSSION

We demonstrated here that cultured rat RMICs take up PGE2 from the extracellular medium in a time-dependent and concentrative fashion. The substrate selectivity and inhibitor sensitivity of the transport, and the presence of the appropriate hybridizing mRNA and immunoreactive protein, indicate that the prostaglandin transporter PGT is mediating the uptake. Exposure of cultured rat RMICs to hyperosmolarity, either in the form of NaCl or urea, causes a sustained increase in the release of PGE2. This increase is accompanied by a transient increase in COX-2 expression and a sustained reduction in cell-surface PGT expression. The data are consistent with a model in which pericellular PG concentrations are controlled at the levels of both release and uptake.

At present, we can only speculate on the physiological role of PG uptake by RMICs. Medullary interstitial PGE2 suppresses osmotic water reabsorption by the collecting duct (7, 30) and solute resorption by the mTALH (33) and induces vasodilation of medullary vasa rectae (27, 40). The latter buffers vasoconstriction so that blood flow is not completely abrogated in this region. PGE2 uptake by RMICs would presumably lower medullary interstitial PGE2 concentrations, increasing ADH action on the collecting duct and mTALH and decreasing medullary blood flow, all of which would decrease urinary salt and water excretion.

Alternatively or additionally, PGT may play a role in controlling RMIC survival. PGE2 generated by COX-2 in RMICs, particularly in response to hypertonicity, prevents RMIC apoptosis (4, 8, 14–16, 25, 29, 36, 37, 39, 41, 42). To the extent that PGT expression at the plasma membrane of these cells would lower pericellular PGE2 concentrations, PGT would be predicted to be proapoptotic in RMICs. The reduction of cell-surface PGT expression in response to hyperosmolarity, along with induction of COX-2, would be predicted to protect RMICs from osmosality-induced apoptosis.

Hyperosmolarity appears to regulate PGT by altering cell-surface protein expression. Aquaporins (23), epithelial sodium

Fig. 12. Immunoblots illustrating the effect of added NaCl for 24 h (A and C) or 7–10 days (B and C) on expression of immunoreactive COX-2. RMIC were grown for 24 h in media with or without 100 mosmol/kgH2O excess NaCl. Cells lysates were prepared and proteins were separated by SDS-PAGE. Immunoblots prepared from the gels were probed with anti-COX-2 polyclonal antibody. A: representative blot illustrating the effect of incubation in media with excess NaCl for 24 h. B: blot showing the effect of incubation in media containing excess NaCl for 7–10 days on COX-2 expression in RMICs. C: densitometric quantification of the data in A and B. Values are expressed as the fold-increase in immunoreactive COX-2 expression relative to isosmolar controls. Data points are means ± SE of 5 and 3 experiments in A and B, respectively. *P < 0.05.
channels (13), glucose transporters (19, 20), and other transporters reside in a vesicular reservoir that can cycle through the plasma membrane (5, 6, 26). Immunocytochemistry revealed that PGT in cultured RMICs is expressed in cytoplasmic vesicles, a pattern that mimics PGT expression in RMICs in situ (3). Although plasma membrane PGT was below the limit of detection using biochemical (biotinylation) detection methods, it is clearly present at the plasma membrane as demonstrated by tracer uptake measurements. In response to hyperosmolarity, PGT function at the cell surface decreases and immunoreactive protein is further internalized, suggesting that PGT, like other plasma membrane transporters, is regulated by membrane insertion and/or retrieval.

Incubation of RMICs in hyperosmolar media greatly increased the net release of PGE$_2$. Early on, basal as well as ET-1-, A23187-, and AA-stimulated PGE$_2$ release were augmented, an effect accompanied by an increase in COX-2 expression. These data are in accord with several other studies in which dehydration of rats resulting in medullary hyperosmolarity (38), and exposure of cultured rabbit renal interstitial cells to a hyperosmolar environment (15), resulted in increased expression of COX-2 and increased PGE$_2$ synthesis/release.

A new feature of our studies is the observation that, after 7–10 days exposure to hyperosmolarity, both A23187 and endothelin could still stimulate increased net PGE$_2$ release in the hyperosmolar state despite low COX-2 expression. Changes in cell-surface receptors cannot explain the endothelin results, as ET-A receptors are downregulated after exposure to hyperosmolarity (36). It is possible that phospholipase-mediated release of arachidonic acid from membrane stores might be upregulated by long-term hyperosmolarity. However, a more likely explanation for the increase in net PGE$_2$ release is the dramatic reduction in cell-surface activity of PGT at 7–10 days.

These studies add to our understanding of the coordinate control of COX and PGT. Work from our laboratory demonstrated that serum coordinately induces both COX-2 and PGT in cultured Swiss 3T3 fibroblasts (R. Lu and V. L. Schuster, unpublished observations). Additionally, a recent report on the bovine corpus luteum showed that PGT and COX-2 were coordinately regulated during the estrus cycle (2). Presumably, a system that regulates both PG synthesis and PG uptake permits finer tuning of PGE$_2$ signaling at cell-surface receptors compared with induction of either alone.

In summary, cultured RMICs take up PGE$_2$ from the extracellular fluid via the prostaglandin transporter PGT. COX-2 and PGT are coordinately regulated in these cells by extracellular osmolarity. Hypertonicity acutely increases COX-2 expression, but over 7–10 days continued exposure COX-2 returns toward baseline. Despite the reduction in COX expression, PGE$_2$ release remains elevated. Hypertonicity acutely lowers PGT cell-surface expression, which remains suppressed during several days’ exposure to hypertonicity. The combined regulation of COX and PGT by hypertonicity suggests that pericellular PG concentrations are controlled at the levels of both release and uptake in these cells.

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