0026-895X/04/6504-973–978$20.00
MOLECULAR PHARMACOLOGY
Vol. 65, No. 4
2773/1136430
Mol Pharmacol 65:973–978, 2004
Printed in U.S.A.

The Two-Step Model of Prostaglandin Signal Termination: In Vitro Reconstitution with the Prostaglandin Transporter and Prostaglandin 15 Dehydrogenase

Teruhisa Nomura, Run Lu, Michael L. Pucci, and Victor L. Schuster

Departments of Medicine (V.L.S.) and Physiology & Biophysics (T.N., R.L., M.L.P., V.L.S.), Albert Einstein College of Medicine, Bronx, New York

Received June 27, 2003; accepted December 15, 2003

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

Termination of prostaglandin (PG) signaling has been proposed to involve carrier-mediated uptake across the plasma membrane followed by cytoplasmic oxidation. Here, we tested this hypothesis directly by coexpressing the PG uptake carrier prostaglandin transporter (PGT) in various cell types with and without human PG 15 dehydrogenase (PG15DH). In HeLa cells, which express neither PGT nor PG15DH, exogenously added PGE2 or PGF2α were rapidly oxidized to the 13,14-dihydro,15-keto metabolites only when PGT and PG15DH were coexpressed, directly confirming the two-step hypothesis. Cells expressing PG15DH that were broken open formed more PG metabolites than cells in which the PGs could gain access to PG15DH only via PGT. Similar results were obtained using the human prostate cancer cell line LNCaP, in which endogenous PG15DH is induced after exposure to dihydrotestosterone. Because PGT in vivo is expressed in renal collecting duct epithelia, we also expressed PGT in Madin-Darby canine kidney cells grown on filters, where it mediated both the active uptake of PGE2 across the apical membrane and the transepithelial transport of PGE2 to the basolateral compartment. When PG15DH was coexpressed with PGT in these epithelial monolayers, about half of the PGE2 taken up apically was oxidized to 13,14-dihydro,15-keto-PGE2, which in turn exited the cells nondirectionally into both the apical and basolateral compartments. Our data represent reconstitution of the longstanding model of PG metabolism consisting of sequential carrier-mediated PG uptake, cytoplasmic oxidation, and diffusional efflux of the PG metabolite.

Prostaglandins (PGs) represent perhaps the ultimate context-dependent signaling system. A single type of PG signaling molecule (e.g., PGE2) can modulate such diverse functions as intraocular pressure, gastric acid secretion, renal salt and water transport, vascular tone, penile erection, and fever (Narumiya et al., 1999; Breyer and Breyer, 2000). To avoid undesired action at a distance, the signal from PGs must be terminated locally.

Considerable evidence suggests that extracellular PGs are taken up by a carrier-mediated transport process and degraded in the cytosol. Experiments in the 1960s and 1970s showed that, although PGE_1 and PGE2 are readily metabolized by tissues to the corresponding 15-keto-PG, there is little or no PG 15 dehydrogenase (PG15DH) activity in the blood or plasma of humans, dogs, or cats (Ferreira and Vane, 1967; Holmes et al., 1968; Nakano et al., 1971; Willman, 1971; McDonald-Gibson et al., 1972; Golub et al., 1974; Smith et al., 1975; Cozzini and Dawson, 1977b). However, when cells are broken open, an intracellular PG15DH is revealed that is capable of oxidizing many diverse prostanoids (Nakano et al., 1969; Anderson and Eling, 1976).

Similarly, the organic anion transport inhibitor bromcresol green had no effect on PG metabolism by cell-free rat homogenates, but it inhibited PG metabolism by intact tissue (Bito and Baroody, 1975; Bito et al., 1977). Taken together, these data led to a proposed model in which PG metabolic clearance occurs via two steps: 1) selective PG uptake across the plasma membrane and 2) oxidation inside the cell.

Several years ago, our laboratory identified the PG transporter PGT (Kanai et al., 1995). PGT is broadly expressed in many cell types (Kanai et al., 1995; Lu et al., 1996; Pucci et al., 1999a). The strong expression of PGT in the lung suggests that PGT may play a role in the single-pass metabolic clearance of PGs through that vascular bed. Indeed, a comparison of the substrate specificities of cloned rat PGT and those of metabolic PG clearance by the isolated, perfused rat lung reveals striking similarities (Itoh et al., 1996; Schuster et al., 2000). In this study, we tested the two-step PG metab-

This work was supported by National Institutes of Health grant 2R01-DK49688-06 (to V.L.S.).

ABBREVIATIONS: PG, prostaglandin; PGT, prostaglandin transporter; rPGT, rat PGT; PG15DH, prostaglandin 15-dehydrogenase; EIA, enzyme immunoassay; ASA, aspirin; MDCK, Madin-Darby canine kidney; DHT, dihydrotestosterone.

olism model directly by coexpressing PGT and PG15DH in the same cells.

Materials and Methods

Cloning and Characterization of Human PG15DH cDNA. The sequence of human NAD+-dependent PG15DH (Ensor and Tai, 1995) was used to search the database. A human pancreas adenocarcinoma expressed sequence tag clone in the vector pOTB7 was obtained from American Type Culture Collection (Manassas, VA; ATCC 5793242). The cDNA insert was sequenced in both directions and subjected to restriction mapping, followed by sequence analysis using the MacVector and GeneWorks software programs. The cDNA contained the entire coding region (798 base pairs) of the human PG15DH. The total size of the cDNA was 2.6 kilobases. The sequence of the 266 open reading frame was the same as that reported by Ensor and Tai (1995) except for one nucleotide change in the open reading frame at codon 52, CAG, which did not result in an alteration of the deduced amino acid sequence. The full-length human PG15DH cDNA in the vector pOTB7 was subcloned into the vector pCR3.1 at BamHI and XhoI sites and was used for the transient transfection of MDCK Tet-Off cells and HeLa cells.

Transient Expression of Rat PGT and PG15DH in HeLa Cells and Determination of PGE2 Uptakes, Fluxes, and Metabolism. HeLa cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 50 U/ml penicillin, and 50 μg/ml streptomycin. The cells were seeded on 35-mm plastic dishes at 2.0×10^5 cells/dish. Twenty-four hours later, the cells formed about 40% confluent monolayers and were transiently transfected by replacing medium with 1.5 ml of transfection complex containing plasmid DNA (0.5 μg of rPGT cloned in pcDNA3, 0.5 μg of pcDNA3 vector alone, 0.5 μg of full-length human PG15DH cloned in pCR3.1, or both at 0.5 μg of plasmid DNA per dish), Effectene transfection reagent (QIAGEN, Valencia, CA), and cell culture medium with a 1:10 ratio of DNA (micrograms) to the reagent (microliters), according to the manufacturer's protocol. As controls, other sets of dishes were transfected with no plasmid DNA (sham control) or the media were just changed (no treatment control). After 3 h of incubation, the transfection complex was replaced with fresh media.

After another 24 h, the cells were treated for 2 h with 100 μM ASA (Sigma-Aldrich, St. Louis, MO) to inhibit endogenous PG synthesis and release. Thereafter, after rapid washes with Waymouth solution, 1 ml of uptake media (~2 nM [^{3}H]PGE2 in Waymouth solution) was added to the dishes. After 10 min at room temperature, solutions were collected for determination of PGE2 and 13,14-dihydro,15-keto-PGE2 by EIA according to the protocol of the manufacturer (Cayman Chemical, Ann Arbor, MI) and for scintillation counting of ^{3}H. The cells remaining on the dishes were quickly washed twice with ice-cold Waymouth solution, harvested, lysed, and assayed for protein content and for [^{3}H]PGE2 uptake. All release and uptake values were calculated as femtomoles per milligram of protein per 10 min and presented as mean ± S.E.M. from duplicate monolayers for four independent experiments. Comparisons were analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test.

In some cases, broken cell assays were carried out as follows. HeLa cells from clone 2 (below), either as control or transfected with PGT, were grown in 35-mm dishes and scraped into 15-ml tubes in 1 ml of growing medium. After centrifugation at 7000 rpm for 5 min, the cell pellet was washed once with Waymouth buffer in room temperature. Thereafter, all of the buffer was aspirated, and the cell pellet with residual buffer was put through 10 freeze-thaw cycles using alternating dry ice/ethanol and a 37°C water bath. One milliliter of Waymouth buffer containing 1 nM [^{3}H]PGE2 and 1 nM unlabeled PGE2 or 1 nM [^{3}H]PGF2α and 1 nM unlabeled PGF2α was added to the broken cell preparation for 10 min at room temperature. After centrifugation, the aqueous buffer was collected for PGE2 or PGF2α metabolite EIA using procedures described by the manufacturer (Cayman Chemical).

Generation of Human PG15DH-Expressing Stable HeLa Cell Lines and Vector Control Cell Lines. HeLa cells were transfected with 0.5 μg of full-length human PG15DH cloned in pCR3.1 or 0.5 μg of closed pCR3.1 vector per dish in the same manner as the transient expression described above. The cells were passaged 1:5 and 1:10 in selective media containing 400 μg/ml geneticin (G418; Invitrogen, Carlsbad, CA) 24 h after transfection and were maintained in the selective media. After 2 to 3 weeks, resistant colonies were selected and expanded in selective media.

Transient Expression in MDCK Cell Monolayers Grown on Filters. MDCK Tet-Off cells (BD Biosciences Clontech, Palo Alto, CA) were maintained in Dulbecco's modified Eagle's medium containing 10% tetracycline-free fetal bovine serum, 50 U/ml penicillin, 50 μg/ml streptomycin, 1 μg/ml puromycin, and 1 μg/ml doxycycline. The cells were seeded on tissue culture inserts (anopore filter, 25 mm; pore size, 0.2 μm; Nalge Nunc International, Naperville, IL) at 4.0×10^5 cells/insert without doxycycline (BD Biosciences Clontech). The day after splitting, when the cells were approximately 40% confluent, they were transiently transfected by replacing apical media with the same transfection complexes as described above for HeLa cells, and the basal media and the apical transfection complexes were changed 2 days after transfection.

The day after changing media, intact monolayers had formed and were assayed for the transepithelial flux of PGE2 and the sided cellular uptake of PGE2 in the cotransfected cells. Two hours before an assay, monolayers were treated with 100 μM ASA. After rapid washing with Waymouth solution, inserts with the monolayers were transferred into six-well plates containing 2 ml of Waymouth solution. To the upper chamber was added 1 ml of medium containing 2 nM [^{3}H]PGE2 and [^{14}C]mannitol as a marker for extracellular trapping as described previously (Endo et al., 2002). After 10 min of incubation at 37°C, solutions were collected from both chambers, followed by one rapid washing with ice-cold 0.5% bovine serum albumin in Waymouth solution and one additional washing with 10 ml of ice-cold Waymouth solution. The collected solutions were subjected to determination of both PGE2 and 13,14-dihydro,15-keto-PGE2 using EIA and to scintillation counting of ^{3}H and ^{14}C. In those experiments, in which EIA for PGs was carried out in the presence of mannitol, we confirmed that the latter did not interfere with the EIA. The monolayers were lysed with 1 ml of 0.125% SDS/0.05 N NaOH for both scintillation counting and protein quantification. All release, flux, and uptake values were calculated as femtomoles per milligram of protein per 10 min and presented as mean ± S.E.M. from duplicate monolayers for five independent experiments. Comparisons were analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test.

PGT Expression in the Human Prostate Cell Line LNCaP. The human prostate cell line LNCaP was obtained from American Type Culture Collection and grown with dihydrotestosterone (DHT) as described previously (Tong and Tai, 2000). For expression of PGT, cells were incubated with the transfection complexes for 48 h. Before uptake assays, they were preincubated with 100 μM ASA for 2 h. Thereafter, the uptake of PGE2, the extraction of PGE2, and the appearance of 13,14-dihydro,15-keto-PGE2 were assayed as described above.

Results

We first set out to test directly the longstanding hypothesis that PG metabolism is a two-step process requiring sequential carrier-mediated uptake followed by cytoplasmic oxidation (Schuster, 1998). HeLa cells grown on plastic were transfected with the rat PGT cDNA with or without full-length human PG15DH cDNA. Cell monolayers were then incubated in [^{3}H]PGE2. At times 0 and 10 min, the incubation

solution was sampled by EIA for remaining PGE2 and for the appearance of the PG metabolite 13,14-dihydro,15-keto-PGE2. After washing, the cell monolayer was harvested and assayed for [^{3}H]PGE2 uptake by scintillation counting.

Figure 1 shows that expression of PGT alone in HeLa cells resulted in modest extraction of PGE2 from the medium and a corresponding increase in PGE2 retention by the cells but little or no appearance of 13,14-dihydro,15-keto-PGE2. These results are similar to those reported previously from our group using a different transient expression vector in HeLa cells (Kanai et al., 1995). Expression of PG15DH alone resulted in essentially no reduction of medium PGE2, no cellular uptake of PGE2, and no production of 13,14-dihydro,15-keto-PGE2. Transfection with 0.5 μg of pcDNA3 vector alone produced results indistinguishable from sham or no transfection. Cotransfection with rPGT and the pcDNA3 vector failed to increase the appearance of PGE2 metabolite, suggesting that adding transfection with PG15DH to that of rPGT did not change the transfection efficiency of the latter. The important finding of Fig. 1 is that coexpression of PGT and PG15DH resulted in a marked extraction of PGE2 from the medium that was accompanied by little intracellular PGE2 accumulation but a large increase in the appearance of free 13,14-dihydro,15-keto-PGE2.

In separate experiments, we created HeLa cells lines stably expressing PG15DH. Figure 2 shows that, as with the transient expression, only the combination of PGT and PG15DH resulted in the appearance of PGE2 metabolite in the medium. Qualitatively similar results were obtained using PGF2α, another substrate of PGT. Transfection of clone 2 PG15DH-expressing cells with PGT increased the appearance of the metabolite 15-keto PGF2α over that obtained in PG15DH-expressing cells devoid of PGT (360 $\pm$ 27 versus 194 $\pm$ 16 fmol/mg protein/10 min, respectively).

We surmise that the three clones shown have differences in PG15DH expression. All three clones extract PGE2 from the medium to about the same degree; i.e., all are transiently transfected about equally with rPGT. In contrast, clone 1 seems to have a relatively low level of PG15DH expression; therefore, the PGE2 that is extracted from the medium is mostly trapped inside ("uptake") and relatively little appears in the medium as the metabolite. Clone 2 seems to have the highest PG15DH expression and so traps little PGE2 in the cytoplasm (uptake), instead converting it rapidly to the metabolite. Clone 5 seems to be intermediate between the two. We interpret these results to mean that PG entered the cytoplasm via PGT, was rapidly converted to the metabolite by cytoplasmic PG15DH, and exited by diffusion with little or no trapping within the cells.

To further explore this idea, we carried out experiments comparing PG metabolism in intact versus broken cells. Clone 2 HeLa cells (expressing PG15DH), or the cytoplasm derived from those cells, were exposed to the PGE2 or PGF2α for 10 min, and the appearance by EIA of 13,14-dihydro,15-keto-PGE2 or 13,14-dihydro,15-keto-PGF2α was determined. As shown in Fig. 2B, intact cells expressing both PG15DH and PGT generate PGE2 and PGF2α metabolites as discussed above (Fig. 2B, smallest black bars). However, when the cells were broken, much more metabolite was formed (Fig. 2B, center bars); we presume that this is because the PGs had greater access to cytoplasmic PG15DH. The presence or absence of cotransfected PGT made no difference in the formation of metabolite by the broken cell preparation (right-hand bars), indicating that PGT does not increase the effectiveness of PG15DH, for example by stabilizing a reactive intermediate state. In the aggregate, these data confirm the longstanding hypothesis that both a plasma membrane

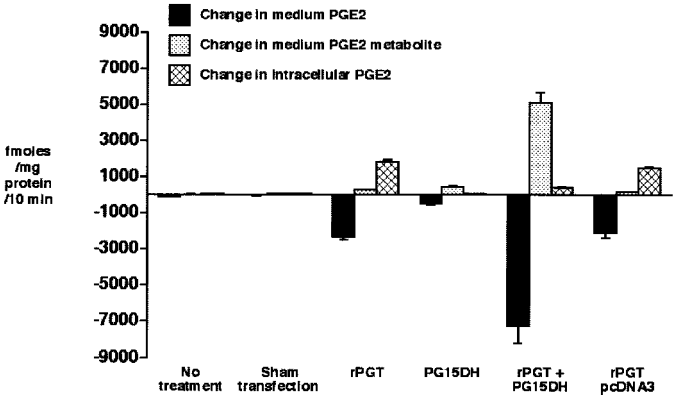

Fig. 1. Reconstitution of the two-step model for PG signal termination in HeLa cells. Transient expression of PGT alone resulted in PGE2 uptake by the cells but no PG metabolism. In contrast, combined expression of PGT and exogenous PG15DH caused marked PGE2 uptake with conversion to the 13,14-dihydro,15-keto-PGE2 metabolite, which appeared in the medium.

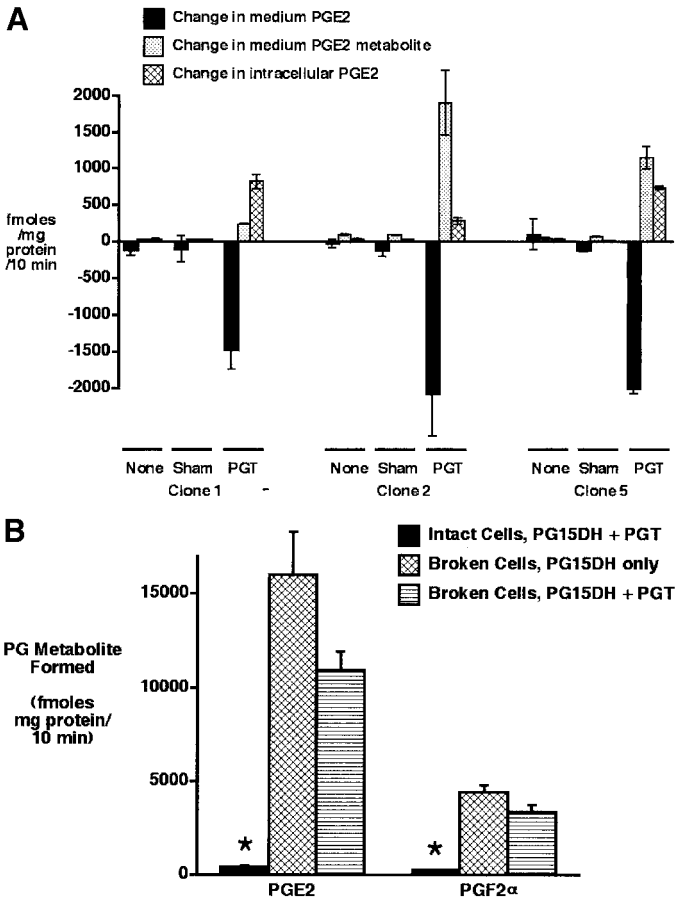

Fig. 2. A, expression of PGT in HeLa cell lines (clones 1, 2, and 5) stably expressing PG15DH. Experiments were carried out as described for Fig. 1. B, metabolism of PGE2 and PGF2α by intact cells versus broken cells. Breaking the cells provides the PGs much greater access to PG15DH than is the case in intact cells expressing cell-surface PGT. $\star$, $p < 0.01$ compared with either of the two broken cell preparations.

uptake carrier (PGT) and a cytoplasmic PG oxidase (PG15DH) are required for PG inactivation.

It could be argued that overexpression of exogenous PG15DH introduces the potential for cellular missorting of the enzyme. To test this model further, we used a cell type that expresses endogenous PG15DH. In the human prostate cancer cell line LNCaP, PG15DH activity is induced by DHT (Tong and Tai, 2000). We transiently transfected LNCaP cells with rPGT, added PGE2, and assayed for the appearance of the metabolite 13,14-dihydro,15-keto-PGE2 and for cellular PGE2 retention. Figure 3 shows that the combination of DHT-induced LNCaP cells and PGT resulted in the uptake of PGE2 from the buffer solution and the appearance of 13,14-dihydro,15-keto-PGE2 in the medium. LNCaP cells not induced with DHT exhibited little ability to release 13,14-dihydro,15-keto-PGE2 in the presence of PGT (data not shown). Again, transfection with 0.5 μg of pcDNA3 vector alone produced results indistinguishable from sham or no transfection (data not shown). Thus, the two-step model holds true with both exogenous and endogenous PG15DH.

Endogenous PGT is expressed in many cell types. In the kidney, PGT is prominently expressed in collecting duct principal cells, either at the apical plasma membrane or in subapical vesicles (Bao et al., 2002). We tested whether such polarized epithelial cells could metabolize PGE2 in the same manner as the HeLa and LNCaP cells above. Toward this end, we previously reported that stable expression of PGT in MDCK monolayers using a constitutive promoter induced a large apical-to-basolateral (absorptive) PGE2 flux across the monolayer, with some intracellular retention of PGE2 (Endo et al., 2002). Figure 4, A and B shows validation of transient PGT expression in MDCK cells in that this approach also resulted in transepithelial [^{3}H]PGE2 flux and cellular accumulation via the apical route. The basal-to-apical flux of [^{3}H]PGE2 was minimal (16 $\pm$ 2 fmol/mg protein/10 min in monolayers transfected with rPGT and 14 $\pm$ 3 fmol/mg protein/10 min in monolayers transfected with rPGT + PG15DH), yielding large net absorptive PGE2 fluxes (561 and 589 fmol/mg protein/10 min, respectively). This is in agreement with our previously published findings using MDCK cells lines stably transfected with rPGT (Endo et al., 2002).

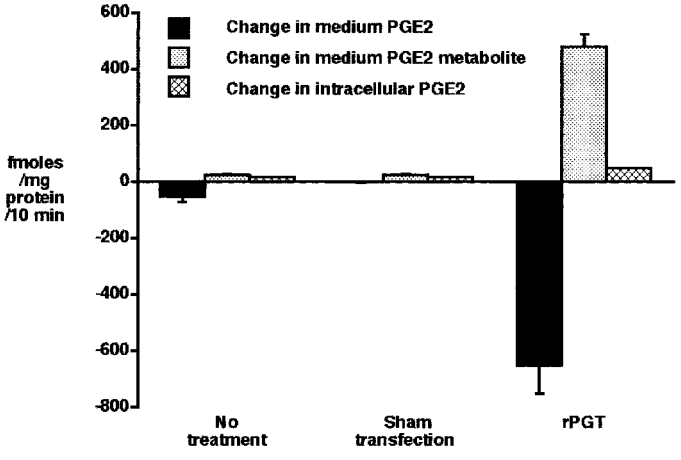

Fig. 3. Reconstitution of the two-step model for PG signal termination in the human prostate cancer cell line LNCaP. Experiments were carried out as described for Fig. 1, except that LNCaP cells induced with DHT to express endogenous PG15DH were used. When DHT-primed LNCaP cells were transfected with PGT, they took up PGE2 from the medium and released 13,14-dihydro,15-keto-PGE2.

Although Fig. 4 indicates that coexpression of PG15DH did not change the overall transepithelial transport of the tritium label, the identity of the radioactivity appearing in the basolateral compartment was not resolved in these experiments. To approach the issue of prostanoid metabolism en route through the cell, we added PGE2 to the apical buffer solution of the MDCK monolayers and monitored over 10 min: 1) the disappearance of PGE2 from the apical solution, 2) the appearance of native PGE2 in the basolateral (trans) solution, and 3) the apical/basal appearance of the PGE2 metabolite 13,14-dihydro,15-keto-PGE2.

Figure 5 shows that expression of PGT alone markedly increased the transepithelial flux of PGE2 but that PGT alone caused little or no 13,14-dihydro,15-keto-PGE2 to appear in either the apical or basolateral medium. In contrast, when PG15DH was coexpressed with PGT, there was a decrease in the appearance of PGE2 in the basolateral solution and an increase in the appearance of 13,14-dihydro,15-keto-PGE2 in both compartments. The simplest interpretation of these results is that PGE2 entered the cell via PGT and underwent oxidation by cytoplasmic PG15DH, whereupon the metabolite exited the cell nondirectionally by simple diffusion (Schuster, 2002).

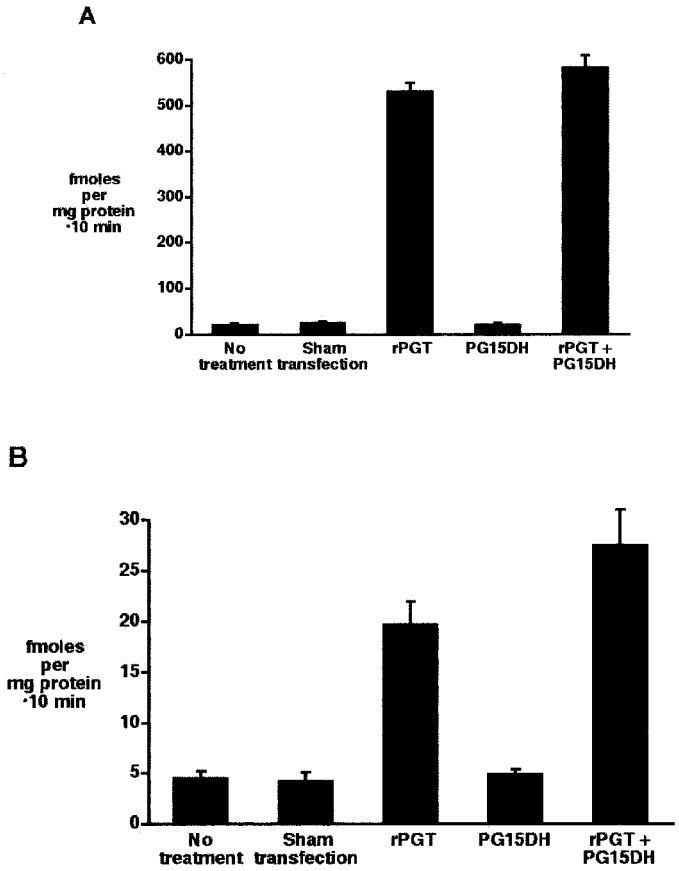

Fig. 4. Transient expression of rat PGT, with or without human PG15DH, in wild-type MDCK cell monolayers grown on permeable filters. A, apical-to-basal [^{3}H]PGE2 flux. B, [^{3}H]PGE2 cellular uptake. Absorptive transepithelial PGE2 flux and uptake of PGE2 from the apical solution were observed only when PGT was expressed.

Discussion

By demonstrating that coexpression of PGT and PG15DH results in PG oxidation, we have directly reconstituted the longstanding two-step model for metabolism and termination of prostanoid signaling. Expression of PG15DH alone in otherwise null cells is insufficient to result in oxidation of PGE2. Rather, coexpression of the PG uptake carrier PGT along with PG15DH is required for PG oxidation. Presumably, this combination permits PG entry into the cell, followed by cytoplasmic oxidation (breaking the cells also permits access of PGs to the cytoplasmic PG15DH). The combination of plasma membrane uptake carrier and cytoplasmic oxidase results in PG oxidation when reconstituted with either exogenous PG15DH (HeLa cells and MDCK cells) or endogenous PG15DH (LNCaP cells).

The affinity of PGT for its substrates is quite high: K_m values are 50 to 95 nM for PGE1, PGE2, and PGF2α. PG concentrations as presented to the transporter in tissues seem to be in this range. In human lung, for example, the PGE2 concentration is approximately 25 ng/g tissue or ~70 nM (Schuster, 1998).

Historically, the two-step model was derived from the observation that PG oxidation in intact cell preparations is blocked by organic anions such as bromcresol green or indocyanine green, whereas these inhibitors are ineffective in broken cell preparations. It was therefore proposed that termination of PG signaling occurs via sequential carrier-mediated PG uptake and cytoplasmic oxidation (Schuster, 1998).

We identified the membrane carrier PGT, which is probably responsible for the PG uptake step (Kanai et al., 1995). PGT has broad tissue expression and a high affinity for the common prostanoids (affinity constants of 50–100 nM) (Kanai et al., 1995; Itoh et al., 1996; Schuster et al., 2000). Expression is particularly strong in the lung, a site of first-pass PG metabolic clearance (Schuster, 1998). In a series of studies using the isolated, perfused rat lung, the structural determinants of the PG metabolism pathway were determined (Eling et al., 1977). These determinants accord remarkably well with the substrate specificities of the cloned PGT (Itoh et al., 1996; Schuster et al., 2000).

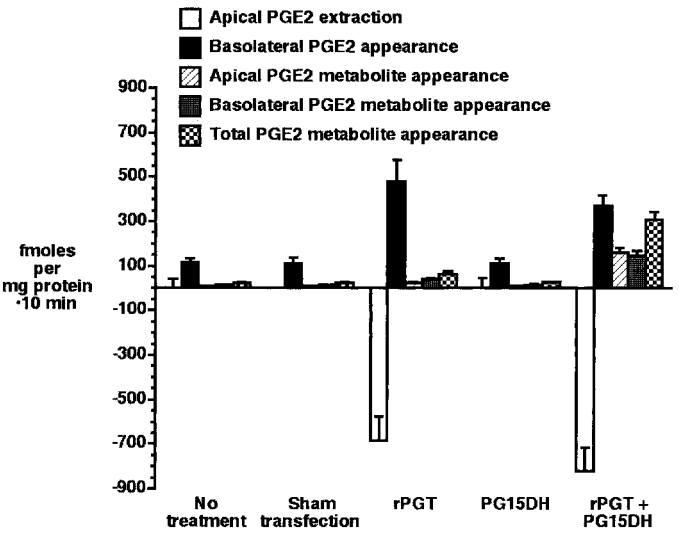

Fig. 5. Metabolism of PGE2 by MDCK cells grown as monolayers on permeable filters. With rPGT expression alone at the apical membrane, PGE2 was extracted from the apical solution and transported to the basolateral compartment without oxidation. In contrast, when PG15DH was coexpressed with PGT, PGE2 was extracted from the apical compartment but was now partly oxidized, the products of which appeared in both compartments.

In agreement with the two-step model, recent data from a targeted knockout of PG15DH showed that the ductus arteriosus failed to close after birth, an effect that is probably caused by an increase in local PGE2 concentrations (Coggins et al., 2002). The data presented here suggest that deletion of PGT might have a similar effect. Studies to test this hypothesis are underway in our laboratory.

It is very likely that the two-step system we have reconstituted here in epithelial monolayers exists in situ. When the rabbit vagina was studied as an isolated, in vitro transporting epithelium with controlled solutions on each side of the preparation, Bito showed that tracer PGF2α exhibited a large mucosa-to-serosa flux but a negligible serosal-to-mucosal flux; eversion of the vaginal sac reversed the direction of the fluxes (Bito, 1975). These data clearly indicate active transport. As in the present studies describing PGE2 transport by MDCK cell monolayers, in the studies of rabbit vagina, about one-third of the transported PGF2α was converted to metabolites (Bito and Wallenstein, 1977).

The mechanism of exit of native and oxidized PGs from cells is probably simple diffusion. We have previously used the *Xenopus laevis* oocyte as a model system to show that PGE2 efflux is linear as a function of the outwardly directed gradient across the plasma membrane (Chan et al., 1998), consistent with simple diffusion. Thermodynamic arguments also favor the diffusional efflux over uptake of PGs (Schuster, 2002). In the present studies, the rapid nondirectional appearance of 13,14-dihydro,15-keto-PGE2 in either compartment of the medium after PGT/PG15DH coexpression suggests efflux of this metabolite by simple diffusion. Similarly, published studies using the isolated, perfused lung also showed that, upon infusion of tracer PGE intravenously, the 15-keto-PG and 13,14-dihydro-PG metabolites appear rapidly in the circulation (Samuelsson and Hamberg, 1971; Anderson and Eling, 1976; Cozzini and Dawson, 1977a; Tsunoda et al., 1982; Devereux et al., 1987; Cawello et al., 1994).

PGT can be regulated, at least in vitro, by serum and by changes in extracellular tonicity, depending on the cell type (Pucci et al., 1999b; Lu et al., 2001). This raises the possibility that PG metabolic clearance might be regulated at the level of the uptake carrier. Further studies will be required to test this hypothesis.

In summary, we have reconstituted the two-step model of PG signal termination consisting of sequential carrier-mediated PG uptake followed by cytoplasmic oxidation.

References

Anderson MW and Eling TE (1976) Prostaglandin removal and metabolism by isolated perfused rat lung. *Prostaglandins* **11:**645–677.

Bao Y, Pucci ML, Chan BS, Lu R, Ito S, and Schuster VL (2002) Prostaglandin transporter PGT is expressed in cell types that synthesize and release prostanoids. *Am J Physiol* **282:**F1103–F1110.

Bito LZ (1975) Saturable, energy-dependent, transmembrane transport of prostaglandins against concentration gradients. *Nature (Lond)* **256:**134–136.

Bito LZ and Baroody RA (1975) Inhibition of pulmonary prostaglandin metabolism by inhibitors of prostaglandin biotransport (probenecid and bromcresol green). *Prostaglandins* **10:**633–639.

Bito LZ, Baroody RA, and Reitz ME (1977) Dependence of pulmonary prostaglandin metabolism on carrier-mediated transport processes. *Am J Physiol* **232:**E382–E387.

Bito LZ and Wallenstein MC (1977) Transport of prostaglandins across the blood-

brain and blood-aqueous barriers and the physiological significance of these absorptive transport processes. *Exp Eye Res* **25 (Suppl):**229–243.
Breyer MD and Breyer RM (2000) Prostaglandin E receptors and the kidney. *Am J Physiol* **279:**F12–F23.
Cawello W, Schweer H, Muller R, Bonn R, and Seyberth HW (1994) Metabolism and pharmacokinetics of prostaglandin E1 administered by intravenous infusion in human subjects. *Eur J Clin Pharmacol* **46:**275–277.
Chan BS, Satriano JA, Pucci ML, and Schuster VL (1998) Mechanism of prostaglandin E2 transport across the plasma membrane of HeLa cells and *Xenopus* oocytes expressing the prostaglandin transporter "PGT". *J Biol Chem* **273:**6689–6697.
Coggins KG, Latour A, Nguyen MS, Audoly L, Coffman TM, and Koller BH (2002) Metabolism of PGE2 by prostaglandin dehydrogenase is essential for remodeling the ductus arteriosus. *Nat Med* **8:**91–92.
Cozzini BO and Dawson CA (1977a) Further identification of PGE1 metabolites formed in the cat lung. *Can J Physiol Pharmacol* **55:**311–313.
Cozzini BO and Dawson CA (1977b) The role of the blood in the metabolism of prostaglandin E1 in the cat lung. *Prostaglandins* **13:**587–597.
Devereux TR, Fouts JR, and Eling TE (1987) Metabolism of prostaglandin PGF2 alpha by freshly isolated alveolar type II cells from lungs of adult male or pregnant rabbits. *Prostaglandins Leukotrienes Med* **27:**43–52.
Eling TE, Hawkins HJ, and Anderson MW (1977) Structural requirements for, and the effects of chemicals on, the rat pulmonary inactivation of prostaglandins. *Prostaglandins* **14:**51–60.
Endo S, Nomura T, Chan BS, Lu R, Pucci ML, Bao Y, and Schuster VL (2002) Expression of PGT in MDCK cell monolayers: polarized apical localization and induction of active PG transport. *Am J Physiol* **282:**F618–F622.
Ensor CM and Tai HH (1995) 15-Hydroxyprostaglandin dehydrogenase. *J Lipid Mediators* **12:**313–319.
Ferreira SH and Vane JR (1967) Prostaglandins: their disappearance from and release into the circulation. *Nature (Lond)* **216:**868–873.
Golub MS, Zia PK, and Horton R (1974) Metabolism of prostaglandins A1 and A2 by human whole blood. *Prostaglandins* **8:**13–20.
Holmes SW, Horton EW, and Stewart MJ (1968) Observations on the extraction of prostaglandins from blood. *Life Sci* **7:**349–354.
Itoh S, Lu R, Bao Y, Morrow JD, Roberts LJ, and Schuster VL (1996) Structural determinants of substrates for the prostaglandin transporter PGT. *Mol Pharmacol* **50:**736–742.
Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW, and Schuster VL (1995) Identification and characterization of a prostaglandin transporter. *Science (Wash DC)* **268:**866–869.
Lu R, Kanai N, Bao Y, and Schuster VL (1996) Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA (hPGT). *J Clin Investig* **98:**1142–1149.
Lu R, Pucci ML, Nomura T, and Schuster VL (2001) Characterization and regulation of the prostaglandin transporter PGT in the mouse Swiss 3T3 fibroblast cell line (Abstract). *J Am Soc Nephrol* **12:**A3035.
McDonald-Gibson WJ, McDonald-Gibson RG, and Greaves MW (1972) Prostaglandin E1 metabolism by human plasma. *Prostaglandins* **2:**251–263.
Nakano J, Anggard E, and Samuelsson B (1969) 15-hydroxy-prostaglandin dehydrogenase. Prostaglandins as substrates and inhibitors. *J Biochem* **11:**386–389.
Nakano J, Monatague B, and Darrow B (1971) Metabolism of prostaglandin E1 in human plasma, uterus and placenta, in swine ovary and in rat testicle. *Biochem Pharmacol* **20:**2512–2514.
Narumiya S, Sugimoto Y, and Ushikubi F (1999) Prostanoid receptors: structures, properties and functions. *Physiol Rev* **79:**1193–1226.
Pucci ML, Bao Y, Chan B, Itoh S, Lu R, Copeland NG, Gilbert DJ, and Schuster VL (1999a) Cloning of mouse prostaglandin transporter PGT cDNA: species-specific substrate affinities. *Am J Physiol* **277:**R734–R741.
Pucci ML, Endo S, and Schuster VL (1999b) Regulation of prostaglandin release and reuptake by hypertonicity in renal medullary interstitial cells (Abstract). *J Am Soc Nephrol* **10:**A0295.
Samuelsson B and Hamberg M (1971) On the metabolism of prostaglandins E and E2 in man. *J Biol Chem* **22:**6713–6721.
Schuster VL (1998) Molecular mechanisms of prostaglandin transport. *Annu Rev Physiol* **60:**221–242.
Schuster VL (2002) Prostaglandin transport. *Prostaglandins Other Lipid Mediat* **68–69:**633–647.
Schuster VL, Itoh S, Andrews SW, Burk RM, Chen J, Kedzie KM, Gil DW, and Woodward DF (2000) Synthetic modification of prostaglandin F(2α) indicates different structural determinants for binding to the prostaglandin F receptor versus the prostaglandin transporter. *Mol Pharmacol* **58:**1511–1516.
Smith JB, Silver MJ, Ingerman CM, and Kocsis JJ (1975) Uptake and inactivation of A-type prostaglandins by human red cells. *Prostaglandins* **9:**135–145.
Tong M and Tai HH (2000) Induction of NAD(+)-linked 15-hydroxyprostaglandin dehydrogenase expression by androgens in human prostate cancer cells. *Biochem Biophys Res Commun* **276:**77–81.
Tsunoda S, Jackson EK, Branch RA, and Gerkens JF (1982) Comparison of the pulmonary, hepatic and renal extraction of PGI2 and 6-keto-PGE1. *Eur J Pharmacol* **77:**147–151.
Willman EA (1971) The extraction of prostaglandin E1 from human plasma. *Life Sci* **10:**1181–1191.

Address correspondence to: V. L. Schuster, M.D., Albert Einstein College of Medicine, Belfer 1008, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: schuster@aecom.yu.edu