Prostaglandins mediate autocrine and paracrine signaling over short distances. We used the renal collecting duct as a model system to test the hypothesis that local control of prostaglandin signaling is achieved by expressing inactivation in the same cell as synthesis. Immunocytochemical studies demonstrated that renal collecting ducts in situ express the prostaglandin (PG) synthesis enzyme, cyclooxygenase-1 (COX-1), as well as both components of prostaglandin metabolic inactivation, i.e. the prostaglandin uptake carrier prostaglandin transporter (PGT) and the enzyme 15-hydroxyprostaglandin dehydrogenase. We characterized this system further using the collecting duct cell line Madin-Darby canine kidney (MDCK), which retains COX-2 and prostaglandin dehydrogenase expression but which has lost PGT expression. When we reintroduced PGT, it was correctly sorted to the apical membrane where it altered the sidedness of prostaglandin E2 (PGE2) release, a process we call “vectorial release via sided reuptake.” Importantly, although COX-2 and prostaglandin dehydrogenase are expressed in the same MDCK cell, they must be compartmentalized because even in the presence of excess dehydrogenase newly synthesized PGE2 is released largely unoxidized. However, when PGE2 undergoes first release and then PGT-mediated reuptake, significant oxidation takes place, suggesting that PGT imports PGE2 into the prostaglandin dehydrogenase compartment. Our data are consistent with a new model that offers significant new mechanisms for the fine control of eicosanoid signaling.

Prostaglandins (PGs)\textsuperscript{1} represent an extreme example of context-dependent autocrine or paracrine signaling. A single type of prostaglandin, PGE2, can activate any of four receptor subtypes (EP\textsubscript{1}, EP\textsubscript{2}, EP\textsubscript{3}, and EP\textsubscript{4}) so as to mediate changes in physiological function as diverse as gastric acid secretion, body temperature, intraocular pressure, blood pressure, and airway reactivity (1–3).

Even within a single organ, such as the kidney, PGE2 has diverse effects including afferent arteriolar vasodilatation, reduction of NaCl resorption by the thick ascending limb of Henle, vasodilatation of medullary vasa rectae, and inhibition of osmotic water flow in the cortical collecting duct (2). On a smaller scale, the renal collecting expresses luminal EP\textsubscript{2} receptors, activation of which increases Na\textsuperscript{+} reabsorption and increases water reabsorption, and basolateral EP\textsubscript{4} receptors, activation of which signals the opposite effects (4, 5). Clearly, to achieve the requisite fidelity in PGE2 signaling, rapid inactivation must occur.

PG inactivation involves active uptake into the cell followed by cytoplasmic oxidation (6). Our laboratory identified the prostaglandin transporter PGT (Slc21a2; oatp2A1) (7), which is the lead candidate for the uptake step. Targeted deletion of mouse PGT results in death at postnatal day 1, most likely the result of an inability to inactive circulating PGE2\textsuperscript{2}.\textsuperscript{2} The enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH) has been extensively characterized by others (8). Indeed, we recently reconstituted the two-step model of PG metabolism by co-expressing PGT and PGDH in cells otherwise lacking these elements; in this system, exogenously added PGE2 was efficiently taken up and oxidized to the inactive metabolite (9).

The need for prostanoid inactivation over very short distances raises the possibility that PG synthesis and the PG inactivation coexist in close proximity. In accord with this hypothesis, in the present study we identified the components of both PGE2 synthesis and PGE2 inactivation in the collecting duct and went on to examine whether PG synthesis and inactivation can occur within the same collecting duct cell.

**EXPERIMENTAL PROCEDURES**

\textit{Immunocytochemistry of Endogenous PGDH and PGT in Rat Kidney—}Male Sprague-Dawley rats weighting 180–250 g were anesthetized with Nembutal. Immediately after exteriorization kidney tissue was fixed in 10% neutral buffered formalin for 24 h and then washed with 1× phosphate-buffered saline. Sections 5 μm thick were deparaffinized in xylene, rehydrated through graded ethanols to water, and equilibrated in phosphate-buffered saline. Sections were pretreated for 20 min by steaming in 0.01 M sodium citrate buffer (pH 6.0; Vector Labs, Burlingame, CA). Endogenous peroxidase activity was quenched with 0.3% H\textsubscript{2}O\textsubscript{2} for 30 min; 5% bovine serum albumin and 2% normal goat serum were used to block nonspecific antibody binding.

For PGT, sections were incubated overnight at 4 °C with mouse monoclonal antibody #20 as straight hybridoma supernatatant (10). For PGDH and cyclooxygenase-1 (COX-1), sections were incubated overnight at 4 °C with a rabbit antisierum to PGDH (catalog number 180615, 1:100) or with rabbit anti-mouse COX-1 (catalog number

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\textsuperscript{1} The abbreviations used are: PG, prostaglandin; PGE2, prostaglandin E2; PGT, prostaglandin transporter; PGDH, prostaglandin dehydrogenase; MDCK, Madin-Darby canine kidney; COX, cyclooxygenase.

\textsuperscript{2} H. Y. Chang, M. L. Pucci, J. Locker, R. Lu, and V. L. Schuster, unpublished data.
160109, 1:500), respectively (Cayman Chemical). In all cases, negative controls consisted of omission of the primary antibody. Sections were then incubated with either biotinylated goat anti-mouse IgG or goat anti-rabbit IgG, respectively (Vector Laboratories, 1:500), for 1 h at room temperature, and antibody sites were visualized by incubating in avidin-biotin peroxidase complex solution (ABC complex, Vectastain, Vector Laboratories) and 3,3'-diaminobenzidine (Dako Corp., Caepin, CA). Counter-staining was done with hematoxylin (Harris Hematoxylin, Poly Scientific, Bay Shore, NY). Survey and photomicroscopy were performed using a Zeiss Axioshot microscope at a magnification of ×650.

Release of PGE2 and PGE2 Metabolite from MDCK Monolayers—For immunassays, the levels of PGE2 and 13,14-dihydro-15-keto-PGE2 in collected solutions were determined using the prostaglandin E2 and Biocycle prostaglandin E2 enzyme immunoassay kits according to the manufacturer's protocol (Cayman Chemical). All release values were calculated as fmol per mg of protein per 10 min from duplicate monolayers.

Agonist-induced PGE2 release experiments were performed by washing with Waymouth solution followed by addition of 1 μl bradykinin or 1 unit/ml thrombin in 1 ml of Waymouth solution added to the basolateral side. After 10 min at 37 °C, the release was terminated by collecting the incubation solutions, after which the monolayers were lysed with 0.5% SDS, 0.1 N NaOH for protein assay.

Cloning and Characterization of Human PGDH cDNA and Transient Overexpression in MDCK Cells—The sequence of human NAD+-dependent PGDH (8) was used to search the NCBI database. A human pancreas adenocarcinoma expressed sequence tag clone in the vector pOTB7 was obtained from American Type Culture Collection (Manassas, VA) (ATCC no. 5793242). The cDNA insert was sequenced in both directions and subjected to restriction mapping, followed by sequence analysis using MacVector and Geneworks software programs. The cDNA contained the entire coding region (798 bp) of the human PGDH. The total size of the cDNA was 2.6 kb. The sequence of the 266-amino acid open reading frame was the same as that reported by Ensor except for one nucleotide change at codon 52, CAG, which did not result in an alteration of the deduced amino acid sequence (11). The full-length human PGDH cDNA in the vector pOTB7 was subcloned into the vector pCR3.1 (Invitrogen) at BamHI and XhoI sites and was used for the transient transfection of MDCK Tet-Off cells.

Transient Expression of PGDH in MDCK Tet-Off Cell Monolayers Grown on Filters—MDCK Tet-Off cells were maintained in Dulbecco's modified Eagle's medium containing 10% tetracycline-free fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, 1 μg/ml puromycin, and 1 mg/ml doxycycline. The cells were seeded on Nunc tissue culture inserts at 4.0×10^5 cells/insert with or without doxycycline. Overexpression of PGDH in MDCK Tet-Off cells was confirmed by reverse transcription-PCR using primers based on the cDNA sequence of human PGDH (forward primer, 5'-TGACCGGCGAGATGAGAAGC-3'; reverse primer, 5'-AATGATGGCCGCTTACCTCC-3'). The initial denaturation at 94 °C for 2 min was followed by 40 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s, and extension at 72 °C for 1 min. A final extension at 72 °C was performed for 10 min. The PCR product, which migrated at ~200 bp, was subcloned into the TA cloning vector pCR 2.1 (Invitrogen) and was sequenced from both ends.

Statistical Analysis—Comparisons were analyzed by paired t test, Wilcoxon ranked test, or one-way analysis of variance followed by Bonferroni's multiple comparison test.

RESULTS

Cyclooxygenase, the prostaglandin transporter PGT, and the enzyme PGDH are co-expressed in renal collecting ducts. To test the hypothesis that PG synthesis and PG metabolic clearance may occur in the same cell, we examined renal cortical collecting ducts, a site of COX expression and PGE2 syn-
thesis (13–20). Fig. 1 shows the immunocytochemical localization of COX-1 (Fig. 1A), PGT (Fig. 1B) and PGDH (Fig. 1C), all in the rat cortical collecting duct. COX-1 was expressed in a perinuclear pattern consistent with its known localization to the nuclear envelope (21). PGT was expressed at the apical membrane as reported previously by our laboratory (10). PGDH was expressed throughout the cytoplasm of cortical collecting duct cells. There was also weaker, punctate labeling for PGDH in proximal tubules.

MDCK Cells Are an in Vitro Model System for the Collecting Duct—To study the functional ramifications of the above findings, we used MDCK cells grown on permeable supports. Although of collecting duct origin (22, 23), MDCK cells have lost expression of PGT.3 To reconstitute the endogenous system, we established stable MDCK cell lines in which green fluorescent protein-tagged rat PGT, or a non-functional mutant (R560N) (24), is expressed under the control of a tetracycline-responsive promoter. As with transient PGT expression (25), these cell Tet-controllable cell lines express PGT at the apical membrane, where it mediates the active uptake of PGE2 from the apical medium. In addition, they synthesize PGE2 from COX-2 (see supplemental data).

Net PGE2 Release in Filter-grown MDCK Cells Is Modulated by PGT-mediated Reuptake—To examine whether apical PGT carries out reuptake of endogenous prostaglandins released into the medium, we stimulated the cells with bradykinin (1 μM, 10 min) or thrombin (1 unit/ml, 10 min) (26) and determined net PGE2 release.

Fig. 2A shows that, although the various MDCK clones expressing wild type rat PGT, inactive R560N PGT, or empty Tet-Off vector were each associated with different overall levels of PGE2 release upon bradykinin stimulation, there was no effect of PGT expression (i.e. ± doxycycline) on the total amount of PGE2 released. Similar results were obtained with 1 unit/ml thrombin (data not shown).

Despite the fact that overall PGE2 release was not affected by PGT expression, the sidedness of PGE2 release was altered. Net release of PGE2 into the apical compartment of the filter apparatus, as a fraction of the total PGE2 release, was significantly reduced when PGT expression was induced at the apical plasma membrane by doxycycline withdrawal. This resulted in a significant reduction in the apical-to-basal release ratio (Fig. 2B). Induction of PGT reduced the average apical PGE2 release.

3 T. Nomura, H. Y. Chang, R. Lu, and V. L. Schuster, unpublished observations.
from 1714 to 573 fmol/mg of protein/10 min and increased the average basolateral PGE2 release from 691 to 1307 fmol/mg of protein/10 min. The most likely explanation for these results is that PGT scavenges PGE2 from the apical solution and brings it back into the cell, whereupon it has another chance to exit by diffusion across the basolateral membrane. PGT expression did not change COX expression, a possible alternative explanation (data not shown).

**PG Synthesis and PG Reuptake and Metabolism Are Compartmentalized in MDCK Cells**—The presence of enzymatically active PGDH in a cell that is also engaged in PGE2 synthesis raises the question of whether newly synthesized PGE2 is subject to oxidation before it is released.

To address this question further, we forced the system toward even more oxidation by overexpressing exogenous PGDH. In the presence of excess PGDH, PGT continued to significantly shift the direction of PGE2 release away from the apical and toward the basal side, as was the case without exogenous PGDH, and the combination of PGT and PGDH resulted in an ~4-fold increase in the appearance of 13,14-dihydro-15-keto-PGE2 into the medium (see supplemental data).

A critical element of these experiments is shown in Fig. 4. Bradykinin did not generate significant PGE2 metabolite above baseline unless PGT was also present, even in the presence of excess PGDH. These data argue against significant access of newly synthesized PGE2 to PGDH. Rather, they suggest that access of PGE2 to PGDH requires first the release of PGE2 into the medium, followed by PGT-mediated reuptake into the cell.

**DISCUSSION**

This study addresses the question of whether PG synthesis and PG inactivation co-exist within the same cell. Rat kidney collecting ducts were shown to strongly express COX plus the two components of PG inactivation, namely the PG uptake carrier PGT and the enzyme PGDH. Using the collecting duct cell line MDCK as an *in vitro* model system, we found that agonist-stimulated PG synthesis and release were followed by PGT-mediated PG reuptake and PGDH-mediated oxidation. The reuptake of released PGE2 by apical PGT shifted the polarity of net PGE2 release by the epithelium, an apparently novel mechanism of directing signaling molecules that we have called “vectorial release via sided reuptake.” Data derived from overexpression of PGDH also lead to the new hypothesis that
the COX synthetic pathway must be compartmentalized relative to the PG reuptake/oxidation pathway.

MDCK cells are a good model system for the collecting duct in that they share many common features (22, 23, 27–31). However, whereas collecting ducts express PGT in subapical vesicles or at the apical plasma membrane (10, 32), MDCK cells have lost this expression. Similarly, PGT expression is lost from endothelial cells when cultured, but expression is regained when shear stress is applied (32–35). In MDCK cells, when PGT was reintroduced it was correctly sorted to the apical plasma membrane (see supplemental data) (25).

Different PGE2 receptors that mediate opposite physiological effects are often expressed on a single cell type. For example, cultured osteoblasts express both EP1 and EP4 receptors, which signal opposite effects on DNA synthesis and alkaline phosphatase activity (36). Similarly, the renal collecting duct expresses apical EP1 receptors and basolateral EP4 receptors that signal opposite effects on Na+/H+ and water transport (4, 5).

Since PGE2 likely exits collecting ducts non-directionally by simple diffusion (37, 38), one method for directing PGE2 toward basolateral (abluminal) side (39), sided PG reuptake may be a general system of directing eicosanoids toward one or another receptor population.

A major finding of the present studies is that, even when PGDH is overexpressed, newly synthesized PGE2 does not appear to have significant access to the enzyme unless the PGE2 is first released and then taken up again by PGT. Taken with the other data presented here, the most likely explanation for these results is that the process of PGE2 synthesis/release is compartmentalized away from the process of PGE2 reuptake and oxidation. A release-reuptake model for PG signaling bears a resemblance to synaptic signaling (Fig. 5) in which secreted neurotransmitter is taken up by the cell that released it and then oxidized (e.g. serotonin (40, 41), γ-aminobutyric acid (42), and glutamate (43)).

The concept of PG compartmentalization within the cell is interesting in light of recent data on PG synthases. Although originally thought to occur exclusively in the cytoplasm (44, 45), recent evidence suggests that PGE2 synthase consists of at least two distinct isoenzymes that are expressed in distinct locations within the cell and are coupled to different pools of arachidonic acid (46–48). The mechanisms of this coupling, as well as of the compartmentation described in the present study, are unknown.
The control of net PG release now appears to be more complex than previously appreciated. In addition to control steps at the level of phospholipase, COX, and the PG synthases, PG reuptake is also controlled. Unpublished work from our laboratory indicates that PGT and COX-2 expression in Swiss 3T3 fibroblasts is coordinately regulated by serum. Similarly, a recent report showed that PGT and COX-2 are coordinately regulated during the estrus cycle in the bovine corpus luteum (49). We propose that carrier-mediated PG reuptake represents a level of control that is complementary to the regulation of COX-2.

In summary, we have presented data in support of a new model of eicosanoid signaling in which PG synthesis and PG inactivation occur in the same cell and are segregated from each other.

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