Regulation of prostaglandin EP<sub>1</sub> and EP<sub>4</sub> receptor signaling by carrier-mediated ligand reuptake

Yuling Chi<sup>1</sup>, Sylvia O. Suadicani<sup>2,3</sup> & Victor L. Schuster<sup>1,4</sup>

<sup>1</sup>Department of Medicine, Albert Einstein College of Medicine, Bronx, New York, 10461
<sup>2</sup>Department of Urology, Albert Einstein College of Medicine, Bronx, New York, 10461
<sup>3</sup>Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York, 10461
<sup>4</sup>Department of Physiology & Biophysics, Albert Einstein College of Medicine, Bronx, New York, 10461

Keywords
Eicosanoids, G-protein-coupled receptors, prostaglandins, signal transduction, SLCO2A1

Abstract
After synthesis and release from cells, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) undergoes reuptake by the prostaglandin transporter (PGT), followed by cytoplasmic oxidation. Although genetic inactivation of PGT in mice and humans results in distinctive phenotypes, and although experiments in localized environments show that manipulating PGT alters downstream cellular events, a direct mechanistic link between PGT activity and PGE<sub>2</sub> (EP) receptor activation has not been made. Toward this end, we created two reconstituted systems to examine the effect of PGT expression on PGE<sub>2</sub> signaling via two of its receptors (EP<sub>1</sub> and EP<sub>4</sub>). In human embryonic kidney cells engineered to express the EP<sub>1</sub> receptor, exogenous PGE<sub>2</sub> induced a dose-dependent increase in cytoplasmic Ca<sup>2+</sup>. When PGT was expressed at the plasma membrane, the PGE<sub>2</sub> dose–response curve was right-shifted, consistent with reduction in cell surface PGE<sub>2</sub> availability; a potent PGT inhibitor acutely reversed this shift. When bradykinin was used to induce endogenous PGE<sub>2</sub> release, PGT expression similarly induced a reduction in Ca<sup>2+</sup> responses. In separate experiments using Madin–Darby Canine Kidney cells engineered to express the PGE<sub>2</sub> receptor EP<sub>4</sub>, bradykinin again induced autocrine PGE<sub>2</sub> signaling, as judged by an abrupt increase in intracellular cAMP. As in the EP<sub>1</sub> experiments, expression of PGT at the plasma membrane caused a reduction in bradykinin-induced cAMP accumulation. Pharmacological concentrations of exogenous PGE<sub>2</sub> induced EP<sub>4</sub> receptor desensitization, an effect that was mitigated by PGT. Thus, at an autocrine/paracrine level, plasma membrane PGT regulates PGE<sub>2</sub> signaling by decreasing ligand availability at cell surface receptors.

Abbreviations
DMEM, Dulbecco’s modified Eagle’s medium; EP<sub>1</sub>-HEK, HEK cells stably expressing the PGE<sub>2</sub> receptor EP<sub>1</sub>; EP<sub>2</sub>, prostaglandin E<sub>2</sub> receptor; FBS, fetal bovine serum; Fura-2 AM, fura-2 acetoxymethyl ester; G418, geneticin; GAPDH, glyceraldehyde phosphate dehydrogenase; GFP, green fluorescence protein; GPCR, G-protein-coupled receptor; HEK, human embryonic kidney; HPGD, 15-hydroxyprostaglandin dehydrogenase; HRP, horseradish peroxidase; MDCK, Madin–Darby Canine Kidney; P/S, penicillin/streptomycin; PBS, phosphate-buffered saline; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PGF<sub>2α</sub>, prostaglandin F<sub>2α</sub>; PG, prostaglandin; PGE<sub>2</sub>-HEK, HEK cells stably expressing both the PGE<sub>2</sub> receptor EP<sub>1</sub> and the prostaglandin transporter PGT; PGT-GFP-MDCK, MDCK cells stably expressing GFP-tagged PGT; PGT, prostaglandin transporter; PSF, phenylmethyl sulfonyl fluoride; SLC, solute carrier; T26A, N-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)
Ligand Reuptake Controls Prostaglandin E₂ Signaling

Y. Chi et al.

Introduction

In recent years, mechanisms mediating prostaglandin E₂ (PGE₂) signal termination have become increasingly clear. Following synthesis, release, and binding to its cognate receptors (EP₁-₄), PGE₂ is metabolically inactivated by a two-step process, consisting first of energetically active uptake across the plasma membrane by the prostaglandin transporter (PGT) (SLCO2A1) (Kanai et al. 1995), and then cytoplasmic oxidative inactivation by 15-OH prostaglandin dehydrogenase (HPGD) (Tai et al. 2002). PGE₂ influx across the plasma membrane by PGT is rate limiting for this inactivation, because heterologous expression of HPGD without PGT is insufficient to result in PGE₂ oxidation (Nomura et al. 2004).

Based on this model, our laboratory has advanced the hypothesis that PGE₂ signaling is akin to synaptic signaling (Nomura et al. 2005). Notably, both neurotransmitters and prostanoids are synthesized by inducible enzymes (Saadat et al. 1989; Smith et al. 2000; Stichtenoth et al. 2001; Murakami and Kudo 2004); both systems involve triggered release of ligand into the extracellular compartment (Greengard 2001; Kudo and Murakami 2002); both sets of G-protein coupled receptors (GPCRs) utilize similar molecular signaling and regulatory mechanisms (Lefkowitz 1993; Neuschafer-Rube et al. 2004); and both systems involve receptors (EP₁-₄), PGE₂ is metabolically inactivated by a two-step process, consisting first of energetically active uptake across the plasma membrane by the prostaglandin transporter (PGT) (SLCO2A1) (Kanai et al. 1995), and then cytoplasmic oxidative inactivation by 15-OH prostaglandin dehydrogenase (HPGD) (Tai et al. 2002). PGE₂ influx across the plasma membrane by PGT is rate limiting for this inactivation, because heterologous expression of HPGD without PGT is insufficient to result in PGE₂ oxidation (Nomura et al. 2004).

Despite the attractiveness of this hypothesis, it has not been shown definitively that PGE₂ reuptake by PGT can control signaling at a local level. Although genetic inactivation of PGT in mice results in patent ductus arteriosus (“EP”) receptors, along with the prostaglandin reuptake carrier PGT, in kidney cell lines. We then acutely manipulated cell surface PGT function and directly measured either signaling through the receptors or ligand-induced receptor desensitization. Our results demonstrate that plasma membrane PGT modulates the concentration of cell surface PGE₂ in an autocrine/paracrine signaling mode. As a result, PGT directly modulates ligand access to, and activation of, EP₁ and EP₄ receptors.

Materials and Methods

Cell lines

Wild-type human embryonic kidney-293 cells (WT-HEK), wild-type Madin–Darby Canine Kidney (WT-MDCK) cells, and MDCK cells expressing green fluorescence protein-tagged PGT (PGT-GFP-MDCK) (Endo et al. 2002) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. HEK cells stably expressing the EP₁ receptor (EP₁-HEK) (Petit-Peterdi et al. 2003) were cultured in F12 medium supplemented with 10% FBS, 1% P/S, 1.2 g/L NaHCO₃, and 0.05 mg/mL G418. We generated a derivative of the EP₁-HEK line stably expressing PGT (PGT-EP₁-HEK) by transfecting EP₁-HEK cells with human PGT cloned into the pcDNA3.1/Hygro (+) vector (Invitrogen, Grand Island, NY), followed by selection with hygromycin (0.25 mg/mL) in F12 media as described for EP₁-HEK.

mRNA quantification

EP₁, PGT, and glyceraldehyde phosphate dehydrogenase (GAPDH) mRNAs in EP₁-HEK cells and in PGT-EP₁-HEK cells were quantified by qRT-PCR using the SYBR system (Invitrogen). The EP₁ (mouse) primers used were: 5'-CATCGGCTAGGCTCAGGTTA-3' (forward) and 5'-A GCAGGCAAGGTTCCAG-3' (backward). The PGT (human) primers used were: 5'-TGTCAGGAGTTGGCA GAGC-3' (forward) and 5'-AGCAGACCTCTACAGC G-3' (backward). The GAPDH (human) primers used were: 5'-AATGAGGGGTCATTTGATG-3' (forward) and 5'-A AGGTAGGTCGGAGTCAA-3' (backward).

Measurement of PGT function

PGT-mediated ³H-PGE₂ uptake was measured using methods previously described (Kanai et al. 1995; Chi et al. 2006).
Protein extraction and quantification by Western blotting
Protein extraction and quantification by Western blotting were conducted using methods previously described (Syeda et al. 2012). EP1 was probed with an anti-EP1 polyclonal antibody (Cayman Chemical, Ann Arbor, MI) overnight at 4°C, with a goat anti-rabbit IgG horseradish peroxidase (HRP)-conjugated antibody as secondary antibody.

Measurements of intracellular Ca^{2+} transients
Changes in cytosolic Ca^{2+} levels were measured in cells loaded with the ratiometric Ca^{2+} indicator Fura-2 AM (Molecular Probes, Grand Island, NY) and imaged on an epifluorescence microscope as previously described (Suadicani et al. 2004, 2006). Experiments were performed with cells bathed in Tyrode solution ([in mmol/L] 137.0 NaCl, 2.7 KCl, 0.5 MgCl2, 1.8 CaCl2, 12.0 NaHCO3, 0.5 NaH2PO4, 5.5 glucose, and 5 HEPES; pH 7.4). Values of intracellular Ca^{2+} levels were obtained from Fura-2 ratio images using an in vitro calibration curve.

EP4 cell surface expression
WT-MDCK and PGT-GFP-MDCK cells were transiently transfected with a human EP4-expressing cDNA (UMR cDNA Resource Center, www.cdna.org) using lipofectamine reagent (Invitrogen) 48 h after being seeded. At 24 h after transfection, the cells were treated with exogenous PGE2 so as to obtain immediate medium PGE2 concentrations of 0, 10, 20, 30, or 50 nmol/L. These experiments were performed in the absence or presence of the PGT inhibitor T26A (Chi et al. 2011) (5 μmol/L) at 37°C for 10 min. Immediately before harvesting the cells, media were collected to determine the result of contact with the cell monolayers on extracellular PGE2 concentrations. Cells were then collected and lysed with buffer containing 10 mmol/L Tris-HCl, pH 7.4, 1 mmol/L EDTA, 10 mmol/L MgCl2, 0.1 mmol/L PSF, and 20 μmol/L indomethacin to inhibit any further PGE2 synthesis. Cell surface EP4 receptor expression was quantified by [3H]-PGE2 binding as previously described (Nishigaki et al. 1996). Briefly, the harvested cells were homogenized using a Potter–Elvehjem homogenizer in an ice-cold lysing buffer. The homogenate was centrifuged at 10,000 g for 5 min, and the supernatant was further centrifuged at 19,500 rpm for 45 min at 4°C. The pellet was washed once and then resuspended in 100 μL buffer A (20 mmol/L HEPES-NaOH, pH 7.4, 1 mmol/L EDTA, 10 mmol/L MgCl2 containing 4 nmol/L [3H]-PGE2 (170,000 dpm) and incubated at 30°C for 1 h. One milliliter cold buffer A was then added to stop binding. The mixture was filtered through a glass fiber which was then transferred to a scintillation vial. Ten milliliter scintillation solution was added to the vial and [3H]-PGE2 was counted by scintillation counting. Nonspecific binding was determined using a 1000-fold excess of unlabeled PGE2 in the incubation mixture. The specific binding was calculated by subtracting the nonspecific binding value from the total binding value.

PGE2 measurement
Extracellular PGE2 concentrations were quantified using an enzyme-linked immunoassay kit (Cayman Chemical) according to the manufacturer’s protocol.

cAMP measurement
WT-MDCK and PGT-GFP-MDCK cells were transfected with the EP4-expressing vector 24 h after being seeded onto six-well plates. Twenty-four hours after transfection, they were incubated with the cAMP/cGMP phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 250 μmol/L) for an additional 18 h. Cells were then stimulated either with 10 μmol/L bradykinin or with various concentrations of exogenous PGE2 (0, 10, 20, 30, or 50 nmol/L), in the absence or presence of T26A (5 μmol/L), at 37°C for 30 and/or 10 min, respectively. Thereafter, cells were washed twice with phosphate-buffered saline (PBS) and were lysed with PBS containing 0.1 mol/L HCl and 0.1% triton-X 100 (250 μL/well) at room temperature for 15 min. Cell lysates were centrifuged (10,000g, 4°C for 10 min), and cAMP in the supernatants was measured using an enzyme-linked immunoassay (ELISA) kit from Cayman Chemical according to the manufacturer’s protocol.

Results

Generation of a cell line coexpressing PGT and EP1
To investigate functional interplay between the PGT transporter and EP1 receptors, we began with a cell line that stably overexpresses the mouse EP1 receptor, “EP1-HEK” (Peti-Peterdi et al. 2003). From this, we generated a new cell line, “PGT-EP1-HEK,” which stably overexpresses both mouse EP1 and human PGT. Figure 1A shows that PGT mRNA expression in PGT-EP1-HEK cells is about 140-fold that of EP1-HEK cells. Figure 1B shows that PGT-EP1-HEK cells exhibit significant tracer PGE2 uptake, whereas EP1-HEK cells exhibit almost no PGE2 uptake. Figure 1C and D show that EP1 mRNA and protein expression in PGT-EP1-HEK cells were not different from that in the original EP1-HEK cells.
PGT modulates EP₁-mediated Ca²⁺ release in response to exogenously applied PGE₂

EP₁ receptor activation triggers intracellular Ca²⁺ release (Sugimoto and Narumiya 2007). Indeed, in both EP₁-HEK and PGT-EP₁-HEK cells, activation of EP₁ by exogenous PGE₂ (0.3 nmol/L to 3 μmol/L) induced a concentration-dependent rise in intracellular Ca²⁺ levels (Fig. 2A). However, in PGT-EP₁-HEK cells, which express the PGT reuptake carrier, the dose–response curve for
PGE$_2$ was significantly right-shifted (Fig. 2A). The EC$_{50}$ (half-maximal effective concentration) value for PGE$_2$ in EP$_1$-HEK cells was 12.8 nmol/L (95% CI = 7.2–22.8 nmol/L, n = 37 cells), whereas in PGT-EP$_1$-HEK cells it was 86.9 nmol/L (95% CI = 51.7–146.0 nmol/L, n = 211 cells) (P < 0.01). Of note, this rightward shift of the PGE$_2$ dose–response curve cannot be attributed to differing levels of EP$_1$ expression (see Fig. 1C and D). The maximal response to PGE$_2$ was slightly but not significantly lower in PGT-EP$_1$-HEK compared to EP$_1$-HEK cells (Fig. 2A).

Acute inhibition of PGT transport in PGT-EP$_1$-HEK cells with T26A (5 µmol/L) resulted in significantly higher maximal Ca$^{2+}$ responses to PGE$_2$ (P < 0.05) compared to nontreated PGT-EP$_1$-HEK cells (Fig. 2A). T26A shifted the PGE$_2$ dose–response curve directionally, albeit not statistically significantly, back toward that of EP$_1$-HEK cells (EC$_{50}$ = 51.8 nmol/L; 95% CI = 36.3–73.9 nmol/L, n = 324). Given the known effect of PGT to mediate PGE$_2$ uptake from the extracellular compartment (Nomura et al. 2004), the data of Figure 2A suggest that, at any given concentration of PGE$_2$ added to the culture medium, plasma membrane PGT lowers the PGE$_2$ concentration at the cell surface, as revealed by a reduction in EP$_1$ receptor activation.

To test this hypothesis further, we added exogenous PGE$_2$ to EP$_1$-HEK and PGT-EP$_1$-HEK cell monolayers so as to achieve initial PGE$_2$ concentrations of 0, 10, 20, 30, and 50 nmol/L. We then measured PGE$_2$ concentrations in the bathing media after 10 min exposure to the monolayers. As shown in Figure 2B, in EP$_1$-HEK cell monolayers, the medium PGE$_2$ concentrations at 10 min were not different from the initial concentrations, indicating that, in the absence of PGT, the added PGE$_2$ remained in the extracellular compartment. Treatment with T26A (5 µmol/L) did not affect medium PGE$_2$ levels in EP$_1$-HEK cells. However, in PGT-EP$_1$-HEK cells, the medium PGE$_2$ concentration after 10 min exposure to the monolayer was significantly reduced compared to that of control cells and, in these cells, acute inhibition of PGT with T26A returned medium PGE$_2$ levels at 10 min to levels similar to those in EP$_1$-HEK cells (Fig. 2B). Taken together, the data of Figure 2 indicate that PGT expression at the cell membrane affects both PGE$_2$ availability for signaling at the cell surface as well as the bulk-fluid phase PGE$_2$ concentration.

**PGT controls endogenous PGE$_2$ bioavailability and modulates autocrine/paracrine PGE$_2$ signaling via the EP$_1$ receptor**

Cells synthesize and release endogenous PGE$_2$ in response to diverse stimuli, such as bradykinin (Miller 2006). We have shown previously that PGT modulates the net release of PGE$_2$ into the bulk cell culture medium in MDCK cells in response to bradykinin (Nomura et al. 2005). Here, we confirmed these results in HEK cells. We treated EP$_1$-HEK and PGT-EP$_1$-HEK cells with bradykinin (10 µmol/L) in the absence and presence of the PGT blocker T26A (5 µmol/L). As shown in Figure 3A, although bradykinin-induced PGE$_2$ release from both EP$_1$-HEK and PGT-EP$_1$-HEK cells, the PGE$_2$ concentration in the medium bathing PGT-EP$_1$-HEK cells was

---

**Figure 2.** PGT modulates PGE$_2$-induced Ca$^{2+}$ responses and medium PGE$_2$ levels. (A) Dose-dependent responses of the increase in intracellular Ca$^{2+}$ concentration induced by PGE$_2$ stimulation of EP$_1$-HEK (n = 37 cells), and of PGT-EP$_1$-HEK cells in the absence (n = 211 cells) and presence of the PGT blocker T26A (5 µmol/L, n = 340 cells). PGT shifted the PGE$_2$/Ca$^{2+}$ dose–response curve to the right; PGT inhibition by T26A reversed this effect (see text for statistical analyses). (B) Final PGE$_2$ concentration in media following 10 min exposure to EP$_1$-HEK or PGT-EP$_1$-HEK monolayers in the presence and absence of T26A (5 µmol/L). PGT expression lowered the medium PGE$_2$ concentration. Values are mean ± SEM (n = 3 for each experiment). *P < 0.05.

© 2014 The Authors. Pharmacology Research & Perspectives published by John Wiley & Sons Ltd, British Pharmacological Society and American Society for Pharmacology and Experimental Therapeutics.
significantly lower than that in the medium bathing EP1-HEK cells. Inhibiting PGT with T26A blocked the effect of PGT to lower PGE2 in the medium (Fig. 3A). These results thus confirm those we reported previously using MDCK monolayers (Nomura et al. 2005).

Next, we examined the ramifications of this PGT effect on autocrine EP1 signaling. Bradykinin induces intracellular Ca2+ release in HEK 293 cells by activating B2 receptors (Kramarenko et al. 2009). All three cell lines (WT-HEK, EP1-HEK, and PGT-EP1-HEK) responded to bradykinin with an increase in intracellular Ca2+ levels, although the amplitude of the response was about three-fold higher (*P < 0.05) in the two cell lines overexpressing the EP1 receptor compared to wild-type cells (Fig. 3B). Thus, most of the bradykinin-induced Ca2+ response in EP1-HEK and PGT-EP1-HEK cells results from signaling through EP1. The amplitude of the Ca2+ response to bradykinin in PGT-EP1-HEK cells doubled when these cells were treated with the PGT blocker T26A (Fig. 3B).

Taken together, the data of Figure 3 indicate that PGT controls the concentration of extracellular PGE2 in this autocrine signaling system, and that the resulting cell surface PGE2 concentration is reflected in the degree of signaling through EP1 receptors.

**PGT modulates autocrine/paracrine signaling through the EP4 receptor**

In addition to Ca2+, another important second messenger for PGE2 is intracellular cAMP, resulting from activation of either the EP2 or EP3 receptor by PGE2. We tested the hypothesis that, as with the EP1 receptor, PGT regulates EP4-mediated autocrine/paracrine signaling. We constructed a cell system consisting of wild-type MDCK cells (“WT-MDCK”) or MDCK cells that were stably transfected with PGT tagged with GFP (green fluorescent protein) at the carboxyl terminus (“PGT-GFP-MDCK”) (Endo et al. 2002). These cell lines were then transiently transfected with an EP4 receptor-expressing cDNA. As above, we stimulated both cell lines with bradykinin so as to induce acute, autocrine/paracrine signaling of EP4 receptors by endogenous PGE2.

As shown in Figure 4A, although bradykinin-induced net release of PGE2 from both cell lines, expression of PGT significantly reduced the extracellular PGE2 concentration compared to control, whereas blocking PGT activity with T26A (5 μmol/L) reversed this reduction. As shown in Figure 4B, bradykinin-induced intracellular cAMP production was significantly lower in PGT-GFP-MDCK cells compared to controls, and T26A treatment reversed this effect. These findings demonstrate that, as with EP1 signaling via intracellular Ca2+, PGT regulates autocrine/paracrine signaling by the EP4 receptor via cAMP.

**PGT modulates homologous desensitization of the EP4 receptor in response to exogenous PGE2**

The EP2, EP3, and EP4 receptors undergo homologous desensitization in response to pharmacological addition of extracellular PGE2 (Negishi et al. 1993; Nishigaki et al. 1996; Ashby 1998). To test the hypothesis that PGT regulates agonist-induced EP4 desensitization, we transiently transfected WT-MDCK cells and PGT-GFP-MDCK cells with the human EP4 receptor cDNA and determined cell surface EP4 expression by a ligand-binding assay in response to exogenous PGE2 (0–50 nmol/L).

As shown in Figure 5A, in WT-MDCK cells (“WT”), exogenous PGE2 caused dose-dependent EP4 desensitization, such that exposure to 50 nmol/L PGE2 induced desensitization of about 50% of cell surface EP4 receptors.
In contrast, in cells expressing PGT ("PGT-MDCK"), PGE₂-induced EP₄ desensitization was significantly abrogated. Although, T26A (5 µmol/L) had no effect on PGE₂-induced EP₄ desensitization in WT-MDCK cells, it inhibited PGT-mediated EP₄ retention on the cell surface.

PGT-mediated modulation of EP₄ desensitization involves PGT-mediated removal of exogenous PGE₂, as shown in Figure 5B. The measured PGE₂ concentration in the medium 10 min after PGE₂ addition to the cell monolayer was significantly lower in PGT-GFP-MDCK cells compared to that in WT-MDCK cells. When PGT
was acutely inhibited by T26A, extracellular PGE$_2$ levels measured in media exposed to PGT-GFP-MDCK cells were not different from those observed using WT-MDCK cells (Fig. 5B).

The downstream signaling consequences of these changes in EP$_4$ receptor desensitization are shown in Figure 5C. In PGT-GFP-MDCK cells, PGE$_2$ increased intracellular cAMP accumulation in a dose-dependent manner to a degree that was significantly greater when PGT was active than when it was inhibited (Fig. 5C).

Together, the data presented in Figure 5 suggest a model in which plasma membrane PGT determines the cell surface PGE$_2$ concentration which, in turn, determines the degree of short-term, agonist-induced desensitization. The degree of desensitization, in turn, determines the degree of intracellular cAMP accumulation in response to exogenous PGE$_2$.

**Discussion**

The present studies show that the PGT modulates PGE$_2$ signaling by altering ligand concentration at cell surface receptors. Our findings provide a molecular mechanism for several recent reports in which acute or chronic manipulation of PGT transporter activity altered downstream physiological events. These include relaxation of tracheal smooth muscle by stimulants of protease-activated receptor-2 (Henry et al. 2005), astrocyte-mediated cerebral vasodilation (Gordon et al. 2008), regulation of preadipocyte aromatase activity by PGE$_2$ (Subbaramaiah et al. 2011), and maintenance of a functional corpus luteum (Lee et al. 2013).

Our results may also provide insight into the mechanisms underlying failure of postnatal closure of the ductus arteriosus in mice (Chang et al. 2010), and of pachydermoperiostosis in humans, both of which result from homozygous null PGT alleles (Busch et al. 2012; Diggle et al. 2012; Sasaki et al. 2012; Seifert et al. 2012; Zhang et al. 2012). Although, as described above, an argument can be made that these phenotypes represent PGE$_2$ acting systemically as a hormone, the present results suggest that at least some of the vascular, skeletal, and/or dermal phenotypes resulting from PGT inactivation may represent abnormal autocrine/paracrine PGE$_2$ signaling.

The ability of the PGT transporter to compete with EP receptors for ligand is dependent on the respective abilities of the transporter and the receptors to bind, and turn over, the ligand. The binding constant of PGE$_2$ to PGT is about 90 nmol/L (Kanai et al. 1995), and that of PGE$_2$ for EP$_1$ and EP$_4$ is in the range of 2–20 nmol/L (Narumiya et al. 1999). Although PGT binds PGE$_2$ more weakly than does EP$_1$ or EP$_4$, it is apparently able to compete for the ligand even when EP receptors are expressed, suggesting that PGT likely turns over PGE$_2$ faster than do the receptors.

EP$_1$ signaling is mediated by intracellular Ca$^{2+}$ release. Regardless of whether the source of PGE$_2$ was endogeneous (i.e., postbradykinin) or exogenous, PGT lowered the amount of cell surface PGE$_2$ and reduced EP$_1$ signaling (Figs. 2, 3). Similarly, PGT reduced EP$_4$-mediated intracellular cAMP accumulation when the source of PGE$_2$ was endogeneous (postbradykinin) (Fig. 4). However, when exogenous PGE$_2$ in pharmacological concentrations was introduced, EP$_4$ exhibited rapid desensitization in accord with prior studies (Nishigaki et al. 1996). In that case, PGT reduced cell surface PGE$_2$ and abrogated EP$_4$ desensitization, resulting in an increase in cAMP formation (Fig. 5). Thus, in the case of EP$_4$ signaling, the net result of competition for ligand between PGT and the EP$_4$ receptor depends on the source, timing, and concentration of the cell surface PGE$_2$.

As above, we have previously drawn an analogy between neurotransmitter release/reuptake at the synapse and PG release/reuptake by cells engaged in autocrine/paracrine PG signaling (Nomura et al. 2005). The present studies allow us to extend this analogy insofar as we now show that PGT, like neurotransmitter reuptake carriers, regulates availability of ligand for its GPCR. The analogy is of further interest in that both neurotransmitter and PG reuptake carriers are regulated by their respective ligands. Thus, dopamine activates the dopamine reuptake carrier through dopamine receptors (Zapata et al. 2007), and the PGT substrate PGE$_2$ inhibits PGT through the FP receptor (Vezza et al. 2001).

In the same way that inhibitors of neurotransmitter reuptake have been useful in the management of psychiatric diseases (Mann 2005), inhibitors of prostaglandin reuptake, by raising endogenous PG levels (Chi et al. 2011; Syeda et al. 2012), might be medicinally applicable for conditions in which exogenous prostaglandins are currently used, such as glaucoma (Stjernschantz 2004), pulmonary artery hypertension (Gomberg-Maitland and Olschewski 2008), and vascular insufficiency (Amendt 2005). Because we found that the PGT inhibitor T26A increased autocrine/paracrine signaling through EP$_1$ and EP$_4$ receptors only in the presence of PGT, it appears that T26A does not interact directly with these two PGE$_2$ receptors. Thus, T26A or its derivatives may offer a small-molecule approach to raising endogenous prostaglandin levels.

Finally, we note that there are currently approximately 120 orphan GPCRs (Civilli 2005; Oh et al. 2006) and 90 orphan SLC transporters (Dahlin et al. 2009; Schlessinger et al. 2010), all with unassigned ligands. The present findings show that regulatory competition between GPCRs and SLC carriers occurs outside of the synaptic cleft, and
thus may represent a more general phenomenon than previously appreciated.

Acknowledgements

We thank David Spray (Albert Einstein College of Medicine) for helpful discussions and Janos Peti-Peterdi (University of Southern California, CA) for the EP1-HEK cells.

Disclosures

None declared.

References

Amendt K (2005). PGE1 and other prostaglandins in the treatment of intermittent claudication: a meta-analysis. Angiology 56: 409–415.

Ashby B (1998). Co-expression of prostaglandin receptors with opposite effects: a model for homeostatic control of autocrine and paracrine signaling. Biochem Pharmacol 55: 239–246.

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

Busch J, Frank V, Bachmann N, Otsuka A, Oji V, Metze D, et al. (2012). Mutations in the prostaglandin transporter SLC02A1 cause primary hypertrophic osteoarthropathy with digital clubbing. J Invest Dermatol 132: 2473–2476.

Cattral MS, Altraif I, Greig PD, Blendis L, Levy GA (1994). Toxic effects of intravenous and oral prostaglandin E therapy in patients with liver disease. Am J Med 97: 369–373.

Chang HY, Locker J, Lu R, Schuster VL (2010). Failure of postnatal ductus arteriosus closure in prostaglandin transporter-deficient mice. Circulation 121: 529–536.

Chi Y, Khersonsky SM, Chang YT, Schuster VL (2006). Identification of a new class of prostaglandin transporter inhibitors and characterization of their biological effects on prostaglandin E2 transport. J Pharmacol Exp Ther 316: 1346–1350.

Chi Y, Min J, Jasmin JF, Lisanti MP, Chang YT, Schuster VL (2011) Development of a high affinity inhibitor of the prostaglandin transporter PGT. J Pharmacol Exp Ther 339:633–641.

Civelli O (2005). GPCR deorphanizations: the novel, the known and the unexpected transmitters. Trends Pharmacol Sci 26: 15–19.

Dahlin A, Royall J, Hohmann JG, Wang J (2009). Expression profiling of the solute carrier gene family in the mouse brain. J Pharmacol Exp Ther 329: 558–570.

Diggle CP, Parry DA, Logan CV, Laissue P, Rivera C, Restrepo CM, et al. (2012). Prostaglandin transporter mutations cause pachydermoperiostosis with myelofibrosis. Hum Mutat 33: 1175–1181.

Drvaric DM, Parks WJ, Wyly JB, Dooley KJ, Plauth Jr WH, Schmitt EW (1989). Prostaglandin-induced hyperostosis. A case report. Clin Orthop Relat Res 246:300–304.

Endo S, Nomura T, Chan BS, Lu R, Pucci ML, Bao Y, et al. (2002). Expression of PGT in MDCK cell monolayers: polarized apical localization and induction of active PG transport. Am J Physiol Renal Physiol 282: F618–F622.

Gether U, Andersen PH, Larsson OM, Schousboe A (2006). Neurotransmitter transporters: molecular function of important drug targets. Trends Pharmacol Sci 27: 375–383.

Gomberg-Maitland M, Olschewski H (2008). Prostacyclin therapies for the treatment of pulmonary arterial hypertension. Eur Respir J 31: 891–901.

Gordon GR, Choi HB, Runnga RL, Ellis-Davies GC, MacVicar BA (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature 456: 745–749.

Greengard P (2001). The neurobiology of slow synaptic transmission. Science 294: 1024–1030.

Henry PJ, D’Aprile A, Sel G, Hong T, Mann TS (2005). Inhibitors of prostaglandin transport and metabolism augment protease-activated receptor-2-mediated increases in prostaglandin E2 levels and smooth muscle relaxation in mouse isolated trachea. J Pharmacol Exp Ther 314: 995–1001.

Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW, Schuster VL (1995). Identification and characterization of a prostaglandin transporter. Science 268: 866–869.

Kramarenko II, Bunni MA, Morinelli TA, Raymond JR, Garmovskaya MN (2009). Identification of functional bradykinin B2 receptors endogenously expressed in HEK293 cells. Biochem Pharmacol 77: 269–276.

Kristensen AS, Andersen J, Jorgensen TN, Sorensen L, Eriksen J, Loland CJ, et al. (2011). SLC6 neurotransmitter transporters: structure, function, and regulation. Pharmacol Rev 63: 585–640.

Kudo I, Murakami M (2002). Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat 68–69: 3–58.

Lee J, McCracken JA, Banu SK, Arosh JA (2013). Intratuerine inhibition of prostaglandin transporter protein blocks release of luteolytic PGF2alpha pulses without suppressing endometrial expression of estradiol or oxytocin receptor in ruminants. Biol Reprod 89: 27.

Lefkowitz RJ (1993). G protein-coupled receptor kinases. Cell 74: 409–412.

Letts M, Pang E, Simons J (1994). Prostaglandin-induced neonatal periostitis. J Pediatri Orthop 14: 809–813.
Mann JJ (2005). The medical management of depression. N Engl J Med 353: 1819–1834.

Miller SB (2006). Prostaglandins in health and disease: an overview. Semin Arthritis Rheum 36: 37–49.

Murakami M, Kudo I (2004). Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. Prog Lipid Res 43: 3–35.

Narumiya S, Sugimoto Y, Ushikubi F (1999). Prostanoid receptors: structures, properties, and functions. Physiol Rev 79: 1193–1226.

Negishi M, Sugimoto Y, Irie A, Narumiya S, Ichikawa A (1993). Two isoforms of prostaglandin E receptor EP3 subtype. Different COOH-terminal domains determine sensitivity to agonist-induced desensitization. J Biol Chem 268: 9517–9521.

Neuschafer-Rube F, Hermosilla R, Rehwald M, Ronnstrand L, Schulein R, Wernstedt C, et al. (2004). Identification of a Ser/Thr cluster in the C-terminal domain of the human prostaglandin receptor EP4 that is essential for agonist-induced beta-arrestin1 recruitment but differs from the apparent principal phosphorylation site. Biochem J 379: 573–585.

Nishigaki N, Negishi M, Ichikawa A (1996). Two Gs-coupled prostaglandin E receptor subtypes, EP2 and EP4, differ in desensitization and sensitivity to the metabolic inactivation of the agonist. Mol Pharmacol 50: 1031–1037.

Nomura T, Lu R, Pucci ML, Schuster VL (2004). The two-step model of prostaglandin signal termination: in vitro reconstitution with the prostaglandin transporter and prostaglandin 15 dehydrogenase. Mol Pharmacol 65: 973–978.

Nomura T, Chang HY, Lu R, Hankin J, Murphy RC, Schuster VL (2005). Prostaglandin signaling in the renal collecting duct: release, reuptake, and oxidation in the same cell. J Biol Chem 280: 28424–28429.

Oh DY, Kim K, Kwon HB, Seong JY (2006). Cellular and molecular biology of orphan G protein-coupled receptors. Int Rev Cytol 252: 163–218.

Peti-Peterdi J, Komlosi P, Fuson AL, Guan Y, Schneider A, Qi Z, et al. (2003). Luminal NaCl delivery regulates basolateral PG2 release from macula densa cells. J Clin Invest 112: 76–82.

Reese J, Paria BC, Brown N, Zhao X, Morrow JD, Dey SK (2000). Coordinated regulation of fetal and maternal prostaglandins directs successful birth and postnatal adaptation in the mouse. Proc Natl Acad Sci USA 97: 9759–9764.

Ringel RE, Brenner JJ, Haney PJ, Burns JE, Moulton AL, Berman MA (1982). Prostaglandin-induced periostitis: a complication of long-term PGE1 infusion in an infant with congenital heart disease. Radiology 142: 657–658.

Saadat S, Sendtner M, Rohrer H (1989). Ciliary neurotrophic factor induces cholinergic differentiation of rat sympathetic neurons in culture. J Cell Biol 108: 1807–1816.

Sasaki T, Niizeki H, Shimizu A, Shiohama A, Hirakiyama A, Okuyama T, et al. (2012). Identification of mutations in the prostaglandin transporter gene SLCO2A1 and its phenotype-genotype correlation in Japanese patients with pachydermoperiostosis. J Dermatol Sci 68: 36–44.

Schlessinger A, Matsson P, Shima JE, Pieper U, Yee SW, Kelly L, et al. (2010). Comparison of human solute carriers. Protein Sci 19: 412–428.

Seifert W, Kuhnisch J, Tuysuz B, Specker C, Brouwers A, Horn D (2012). Mutations in the prostaglandin transporter encoding gene SLCO2A1 cause primary hypertrophic osteoarthropathy and isolated digital clubbing. Hum Mutat 33: 660–664.

Smith WL, DeWitt DL, Garavito RM (2000). Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 69: 145–182.

Stichtenoth DO, Thoren S, Bian H, Peters-Golden M, Jakobsson PI, Crofford LJ (2001). Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. J Immunol 167: 469–474.

Stjernschantz J (2004). Studies on ocular inflammation and development of a prostaglandin analogue for glaucoma treatment. Exp Eye Res 78: 759–766.

Suadicani SO, Flores CE, Urban-Maldonado M, Beelitz M, Scemes E (2004). Gap junction channels coordinate the propagation of intercellular Ca2+ signals generated by P2Y receptor activation. Glia 48: 217–229.

Suadicani SO, Brosnan CF, Scemes E (2006). P2X7 receptors mediate ATP release and amplification of astrocytic intercellular Ca2+ signaling. J Neurosci 26: 1378–1385.

Subbaramaiyah K, Hudis CA, Dannenberg AJ (2011). The prostaglandin transporter regulates adipogenesis and aromatase transcription. Cancer Prev Res (Phila) 4: 194–206.

Sugimoto Y, Narumiya S (2007). Prostaglandin E receptors. J Biol Chem 282: 11613–11617.

Syeda MM, Jing X, Mirza RH, Yu H, Sellers RS, Chi Y (2012). Prostaglandin transporter modulates wound healing in diabetes by regulating prostaglandin-induced angiogenesis. Am J Pathol 181: 334–346.

Tai HH, Ensor CM, Tong M, Zhou H, Yan F (2002). Prostaglandin catabolizing enzymes. Prostaglandins Other Lipid Mediat 68–69: 483–493.

Ueda K, Saito A, Nakano H, Aoshima M, Yokota M, Muraoka R, et al. (1980). Cortical hyperostosis following long-term
administration of prostaglandin E1 in infants with cyanotic congenital heart disease. J Pediatr 97: 834–836.

Vezza R, Rokach J, Fitzgerald GF (2001). Prostaglandin F2alpha receptor-dependent regulation of prostaglandin transport. Mol Pharmacol 59: 1506–1513.

Zapata A, Kivell B, Han Y, Javitch JA, Bolan EA, Kuraguntla D, et al. (2007). Regulation of dopamine transporter function and cell surface expression by D3 dopamine receptors. J Biol Chem 282: 35842–35854.

Zhang Z, Xia W, He J, Ke Y, Yue H, Wang C, et al. (2012). Exome sequencing identifies SLCO2A1 mutations as a cause of primary hypertrophic osteoarthropathy. Am J Hum Genet 90: 125–132.