0026-895X/96/040736-07$3.00/0

MOLECULAR PHARMACOLOGY, 50:736–742 (1996).

Structural Determinants of Substrates for the Prostaglandin Transporter PGT

SHIGEKAZU ITOH, RUN LU, YI BAO, JASON D. MORROW, L. JACKSON ROBERTS, and VICTOR L. SCHUSTER

Departments of Medicine, Physiology, and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461 (S.I., R.L., Y.B., V.L.S.), and Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee 37232-6602 (J.D.M., L.J.R.)

Received April 18, 1996; Accepted June 12, 1996

SUMMARY

We recently identified a broadly expressed transporter, PGT, that transports primarily prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$). In the current study, we examined the structural determinants of potential PGT substrates in detail. Rat PGT was transiently expressed in HeLa cells; the timed uptake of tracer PGE_2 was determined in the presence of various concentrations of unlabeled prostanoids; and the resulting inhibitory constants (K_i) were determined by curve-fitting. PGE_2 and $PGF_{2\alpha}$, both known to be transported, had similar affinities for PGT (K_i = 49–50 nM). The strongest interaction (K_i = 13–19 nM) was obtained with prostanoids lacking the 9- or 11-position oxygen groups. A relatively high affinity was also obtained for the bicycloendoperoxides U44069, PGH_2, and U46619 (K_i = 29–39 nM). However, a radioactive representative from this group, U46619, was not transported. Structural modifications that produced a moderately reduced affinity relative to that of PGE_2 (K_i = 56–286 nM) included reduction in C5═C6, the addition of a benzene group at position C18, and isomerization at the C8 position. In complementary studies, tracer isoprostane 8-iso-$PGF_{2\alpha}$ was found to be transported at ~13% the rate of tracer PGE_2. Substantially weaker interaction (K_i = >700 nM) was seen when the 1-position COO^- anionic group was neutralized or when the 15(*S*)—OH group was changed to 15(*R*)—OH or to 15-keto. These results with the cloned rat PGT are very similar to those previously reported in the *in vitro* perfused rat lung and indicate that PGT probably represents the predominant route by which certain prostanoids, including F_2 isoprostanes, are transported across plasma membranes.

PGs and TXs play important roles in human health and disease. After their release from cells, PGs and TXs bind to nearby surface receptors, where they signal a broad array of physiological functions. Because there is no enzymatic activity in the plasma capable of oxidizing PGE_1, PGE_2, or $PGF_{2\alpha}$ (1), these prostanoids could potentially activate receptors at a substantial distance from their sight of release.

Such distal activation does not occur because PGE_1, PGE_2, and $PGF_{2\alpha}$ are cleared in a single passage through any of several vascular beds, including the lung (1–3). In contrast, PGI_2, PGA_1, and PGB_1 are not cleared by the perfused lung preparation (4–8). When lung cells are broken open, the PG dehydrogenase that is released is capable of oxidizing essentially all prostanoids (5, 9). The difference between the nonselective profile of PG metabolism by broken cells, as opposed to the highly selective profile of metabolism by intact cells, indicates that clearance must proceed by selective uptake across the plasma membrane followed by nonselective intracellular oxidation.

We recently identified the first PG transporter cDNA, PGT, which preferentially transports PGE_1, PGE_2, $PGF_{2\alpha}$, and, to a lesser extent, TXB_2 (10). In the rat and human, PGT mRNA is broadly expressed in essentially all tissues examined (10, 11). This broad expression, taken with its substrate selectivity and sensitivity to known blockers of prostanoid transport (10), suggests that PGT may play a primary role in mediating PG clearance from the circulation.

In elegant experiments using the perfused rat lung preparation, Eling *et al.* (6) related several structural features of prostanoids to their pulmonary clearance. Based on those results, we examined the substrate specificity of PGT in more detail. We also examined whether PGT can mediate the transport of isoprostanes, a newly recognized group of prostanoids with considerable potential biological importance (12).

Materials and Methods

Transient expression of PGT in HeLa cells. For HeLa cell expression, cells were grown to 80% confluence onto 35-mm dishes and were infected with recombinant vaccinia virus vTF7-3 (10 plaque-forming units/cell) (13). Thirty minutes after infection, cells were transfected with the full-length PGT cDNA (10 μg/ml) plus Lipofectin (20 μg/ml) as described previously (10, 14). After 3 hr of incubation, vaccinia virus and the DNA/Lipofectin complex were

This work was supported by National Institutes of Health Grant R01-DK49688, the American Heart Association (New York City affiliate), an Irma T. Hirschl-Monique Weill-Caulier Career Scientist Award, and the National Kidney Foundation.

ABBREVIATIONS: PG, prostaglandin; TX, thromboxane; ER, endoplasmic reticulum; oatp, organic anion transporting polypeptide.

removed, and the cells were maintained in Dulbecco's modified Eagle's medium with 5% fetal bovine serum overnight until the uptake assay.

Tracer prostanoid uptake

Uptake in HeLa cells was initiated 19 hr after transfection. Monolayers were washed twice with Waymouth solution, and timed uptake over 5 min at 27° was initiated by adding tracer prostanoid to the Waymouth solution (tracer prostanoid uptake is linear over the initial 5 min; Ref. 10). [^{3}H]PGE_2 (171 Ci/mmol) (DuPont-New England Nuclear, Boston, MA) was added at a concentration of 0.1 μCi/ml/dish (total concentration, ~0.6 nM). [^{3}H]8-Iso-$PGF_{2\alpha}$ (50 Ci/mmol) was commercially prepared as a randomly labeled compound from unlabeled 8-iso-$PGF_{2\alpha}$ (SibTek, Tenafly, NJ) and was added at a concentration of 0.1 μCi/ml/dish (total concentration, ~2.0 nM). Compound purity and specific activity of the tracer 8-iso-$PGF_{2\alpha}$ were determined by mass spectrometry. [^{3}H]U46619 (20.8 Ci/mmol, DuPont-New England Nuclear) was added at a concentration of 0.1 μCi/ml/dish (total concentration, ~5 nM). Tracer uptake was stopped by washing with ice-cold Waymouth solution containing 5% bovine serum albumin once and then four times with Waymouth solution lacking bovine serum albumin. Cells were scraped and counted by liquid scintillation.

For oocyte expression, water or cRNA *in vitro*-transcribed from the full-length PGT cDNA was capped and injected into defolliculated stage V *Xenopus laevis* oocytes (50 ng of mRNA/oocyte). Uptake in oocytes was started 2–3 days after injection by washing three times in ND 96 solution and then adding tracer prostanoid to ND 96 (~0.25 μCi/ml; total concentration, ~1 nM). After timed incubation periods at 27°, oocytes were again washed three times in cold ND 96, were placed in 0.5 ml of 10% sodium dodecyl sulfate, and counted by liquid scintillation. PGT-mediated tracer PG uptake in oocytes is linear over 60 min (15).

Inhibition constants. For substrate K_i determinations, [^{3}H]PGE_2 uptakes were determined in duplicate on a given transfection in the presence of various concentrations (10 nM to 10 μM) of unlabeled prostanoids (Cayman Chemical, Ann Arbor, MI). Inhibition dose-responses were curve-fitted, and the K_i values were calculated for three to five separate transfections. In experiments with PGH_2, particular care was taken to not aliquot the PGH_2 but rather to pipette it directly from the Cayman vial, dilute it serially under nitrogen gas, and apply it immediately to the uptake solution.

Electrostatic potential surface calculations. The molecular electrostatic surfaces of substrates were obtained using Gaussian 92 self-consistent field calculations at the STO-3G level (16). The molecular electrostatic potential surfaces were visualized using AVS Chemistry Viewer programs (Advanced Visual Systems and Molecular Simulations, Waltham, MA). The electron density isosurfaces are displayed at an average electron density of 0.002 e/a_o^3, which corresponds approximately to the van der Waals surface of the molecule (17).

Results

We examined the structural features of prostanoids that account for their interaction with the transporter PGT by determining their ability to compete with tracer PGE_2 for transport. Fig. 1 shows representative inhibition curves for four unlabeled prostanoids. In each case, the data points were fitted by a single exponential curve. The rank order of inhibition by these four compounds was 11-deoxy-PGE_2 > PGE_2 > PGA_1 > $PGF_{2\alpha}$ isopropyl ester (r = 0.999, 0.978, 0.998, and 0.999, respectively; see Table 1). (It should be noted that the standard error bars for $PGF_{2\alpha}$ isopropyl ester overlap 100%, indicating that the mean with this compound is not significantly different than that value.)

Table 1 is a summary of similar inhibition data on 23 unlabeled prostanoids showing the structure of PGE_2 and the structural differences for each prostanoid in relationship to PGE_2. The correlation coefficients are derived from the single-exponential curve-fittings. Apparent inhibitory constants, K_i (in nM), were derived from the fitted curves.

The strongest inhibition (K_i = ~13-19 nM) was obtained with two prostanoids lacking the 9- or 11-position oxygen groups (i.e., 11-deoxy-PGE_2 and 9-deoxy-9-methylene PGE_2). Because no radioactive forms of these compounds were available, we could not determine whether this high affinity represents binding and translocation or only binding.

Fig. 2 (top) shows *ab initio* calculations of electrostatic potential surfaces for PGE_2 compared with 11-deoxy-PGE_2, highlighting the loss of a region of partial electropositivity on the cyclopentane ring of 11-deoxy-PGE_2, and its replacement by a region of electroneutrality on PGE_2. We postulate that this change in surface charge may account for the higher affinity of 11-deoxy-PGE_2 relative to that of PGE_2.

Table 1 shows that the bicycloendoperoxides U44069, PGH_2, and U46619 also exhibit relatively high affinity for the transporter (K_i = ~29-39 nM) compared with PGE_2. In the case of U46619, tracer is available. As depicted in Fig. 3 (*left*),

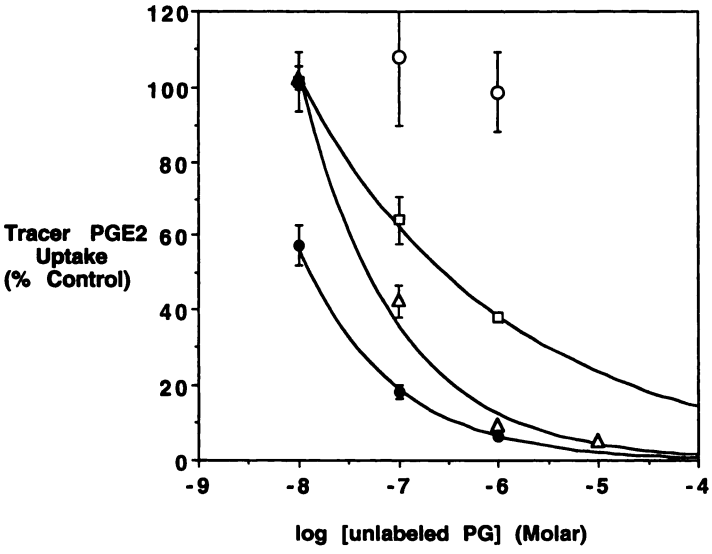
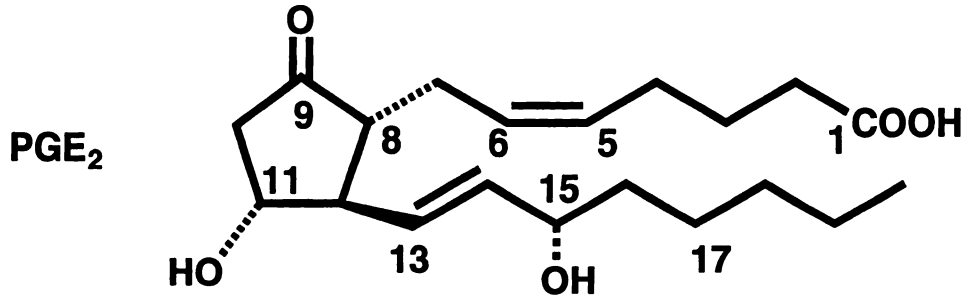


Fig. 1. Inhibition of [^{3}H]PGE_2 uptake by rat PGT transiently expressed in HeLa cells. Various concentrations of unlabeled 11-deoxy-PGE_2, PGE_2, PGA_1, and $PGF_{2\alpha}$ isopropyl ester were added during tracer uptake. *Curves*, exponential fits to the data; correlation coefficients for the curves are given in Table 1.

TABLE 1

Summary of inhibition of tracer [^{3}H]PGE_2 uptake by rat PGT

The structure of PGE_2 is shown for reference.



Compound name	K_i	r	Structural differences relative to PGE_2
	nM		
11-Deoxy-PGE_2	12.9	0.999	$C(11)H_2$
9-Deoxy-9-methylene-PGE_2	18.7	0.954	$C(9)H_2{=}CH_2$
U44069	29.1	0.996	Bicyclo-epoxide: $C(9)H_2{-}O{-}CH_2{-}C(11)H_2$
PGH_2	38.2	0.999	Bicyclo-epoxide: $C(9)H_2{-}O{-}O{-}C(11)H_2$
U46619	39.3	0.949	Bicyclo-epoxide: $C(9)H_2{-}CH_2{-}O{-}C(11)H_2$
PGE_2	48.7	0.978	(Shown above)
$PGF_{2\alpha}$	50.2	0.974	C(9)H—OH
11-β-PGE_2	55.8	0.978	C(11)—OH is in the β configuration
8-Iso-PGE_2	62.3	0.955	C(8) bond is the β stereoisomer
PGB_1	71.7	0.911	$C(5)H_2{-}C(6)H_2$; C(8)=C(12); $C(11)H_2$
PGD_2	88.7	0.944	C(9)H—OH; C(11)=O
PGE_1	95.1	0.897	$C(5)H_2{-}C(6)H_2$
PGD_1	162	0.935	$C(5)H_2{-}C(6)H_2$; C(9)H—OH; C(11)=O
17-Phenyl trinor $PGF_{2\alpha}$	168	0.952	C(9)H—OH; C(17)—phenyl
8-Iso-$PGF_{2\alpha}$	177	0.909	C(8) bond is the β stereoisomer; C(9)H—OH
PGA_1	286	0.998	$C(5)H_2{-}C(6)H_2$; C(10)H=C(11)H
13,14-Dihydro-$PGF_{2\alpha}$	753	0.854	C(9)H—OH; $C(13)H_2{-}C(14)H_2$
15(*R*)-PGE_2	1180	0.660	C(15)—OH is in the β configuration
15-Cyclohexylpentanor $PGF_{2\alpha}$	3460	0.787	C(9)H—OH; C(15)—cyclohexane
$PGF_{2\alpha}$ isopropyl ester	>5000	0.999	C(9)H—OH; $C(1){-}OOCH(CH_3)_2$
15-Keto PGE_2	>5000	0.705	C(15)=O
U51605	>5000	0.483	Diazabicylo: C(9)H—N=N—C(11)H
13,14-Dihydro-15-keto PGE_2	>5000	0.032	$C(13)H_2{-}C(14)H_2$; C(15)=O

although tracer PGE_2 is readily transported in HeLa cells expressing PGT, there is no discernible uptake of tracer U46619. Thus, we can conclude that at least this particular bicycloendoperoxide binds tightly to the transporter but is not translocated by it. On the other hand, the diazabicyclo compound U51605 had essentially no affinity (Table 1).

A number of structural modifications to PGE_2 produced only modest changes in the affinity for PGT. As expected from their similar transport rates (10), PGE_2 and $PGF_{2\alpha}$ had indistinguishable affinities [electrostatic potential surfaces of PGE_2 and $PGF_{2\alpha}$, viewed from the perspective of the cyclopentane ring, are shown in Fig. 2 (*middle*); see Discussion). Isomerization of PGE_2 at the 11-position —OH group also produced essentially no change in affinity.

The 8-position isomers of PGE_2 and $PGF_{2\alpha}$ (C8 bond in β configuration) are members of a family of compounds known collectively as the isoprostanes (12). 8-Iso-PGE_2 had an affinity for PGT ~1.3-fold lower that of the "usual" (α 8-position) PGE_2 (K_i = 62.3 versus 48.7 nM). Similarly, 8-iso-$PGF_{2\alpha}$ had an affinity ~3.5-fold lower than that of $PGF_{2\alpha}$ (177 versus 50 nM). This suggests that 8-iso-PGE_2 and/or 8-iso-$PGF_{2\alpha}$ might represent substrates for the transporter.

This hypothesis was tested directly using tracer 8-iso-$PGF_{2\alpha}$. As shown in Fig. 3 (*right*), after subtracting the background uptakes obtained in the vector controls, the PGT-specific transport rates at the 10-min time points for 8-iso-$PGF_{2\alpha}$ versus PGE_2 were 13.2 and 99.6 pmol/mg of protein/nM substrate, respectively. Thus, tracer 8-iso-$PGF_{2\alpha}$ was transported at ~13% the rate of PGE_2, indicating that 8-iso-$PGF_{2\alpha}$ is definitely a PGT substrate.

Comparison of the affinities of PGE_1 versus PGE_2 and of PGD_1 versus PGD_2 revealed that reduction of C5=C6 approximately doubled the K_i values (95 versus 49 nM and 162 versus 89 nM, respectively; Table 1). Because PGD_2 had a relatively high affinity, we directly tested the transport of tracer PGD_2. As determined in *X. laevis* oocytes, the 30-min uptake of tracer PGE_2 and PGD_2 was 1.39 ± 0.49 fmol/oocyte (10 oocytes) and 1.03 ± 0.25 fmol/oocyte (9 oocytes), respectively. Similar results were obtained in HeLa cells expressing PGT (data not shown). Thus, PGD_2 is a good substrate for rat PGT.

PGB_1 had a fairly high affinity for PGT (K_i = ~72 nM; Table 1), whereas the affinity of PGA_1 was substantially lower (K_i = 286 nM). We did not synthesize tracer versions of either of these prostanoids to directly test transport.

We examined various permutations of the C12—C20 acyl chain (Table 1). The addition of a benzene ring to $PGF_{2\alpha}$ at position C18 (17-phenyl trinor $PGF_{2\alpha}$) produced a modest decrease in affinity. Reduction of the C13=C14 alone (13,14-dihydro-$PGF_{2\alpha}$) fairly markedly decreased the affinity for PGT.

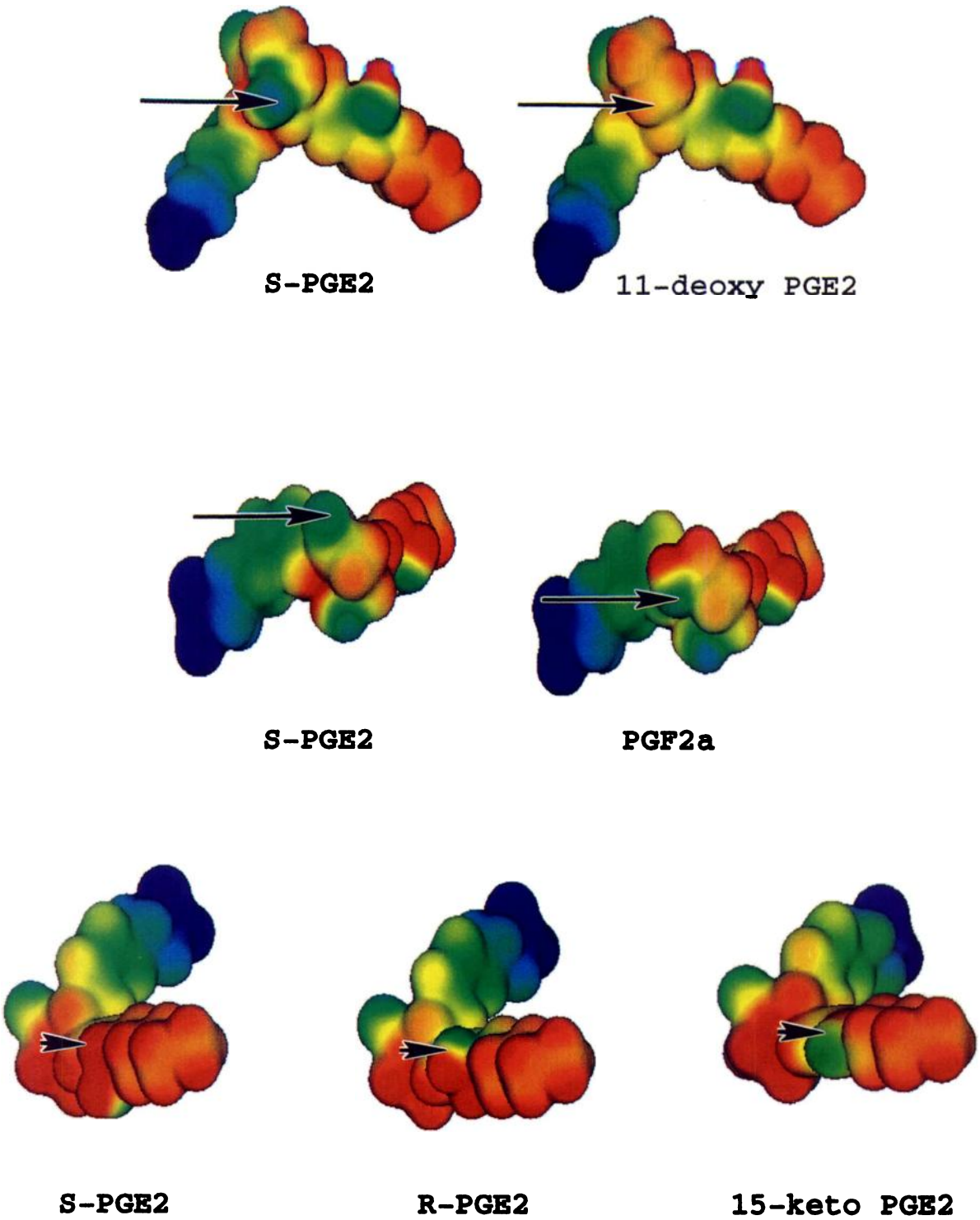


Fig. 2. *Ab initio* electrostatic potential surface models for 15(*S*)-PGE_2 compared with 11-deoxy-PGE_2 (*top*), with $PGF_{2\alpha}$ (*middle*), or with 15(*R*)-PGE_2 and 15-keto-PGE_2 (*bottom*). *Blue*, electron-rich, partial negative charge; *red*, electron-deficient, partial positive charge; *green*, neutral, uncharged; *light blue* and *yellow*, slightly electron-rich and electron-deficient regions, respectively. *Arrows* (*top* and *middle*), shifting position of partial charges between the two molecules. *Arrowheads* (*bottom*), a site of variable electropositivity or electroneutrality on the three substrates.

At the 15 position, the addition of a cyclopentane ring markedly impaired affinity (15-cyclohexylpentanor-$PGF_{2\alpha}$), as did changing the —OH group to a ketone (15-keto-PGE_2 or 13,14-dihydro-15-keto-PGE_2). It is of particular interest that changing the stereochemistry of the 15-position —OH group from the (*S*) to the (*R*) configuration produced a substantial reduction in affinity, from ~49 nM for 15(*S*)-PGE_2 to 1180 nM for 15(*R*)-PGE_2. This is in good agreement with the data of Eling *et al.* (6) using the isolated, perfused rat lung preparation.

Finally, neutralization of the strongly negative 1-position carboxyl group ($PGF_{2\alpha}$ isopropyl ester) eliminated affinity for PGT. This result is consistent with other data from our laboratory indicating that the substrate is recognized by the transporter as the anion (15, 18).

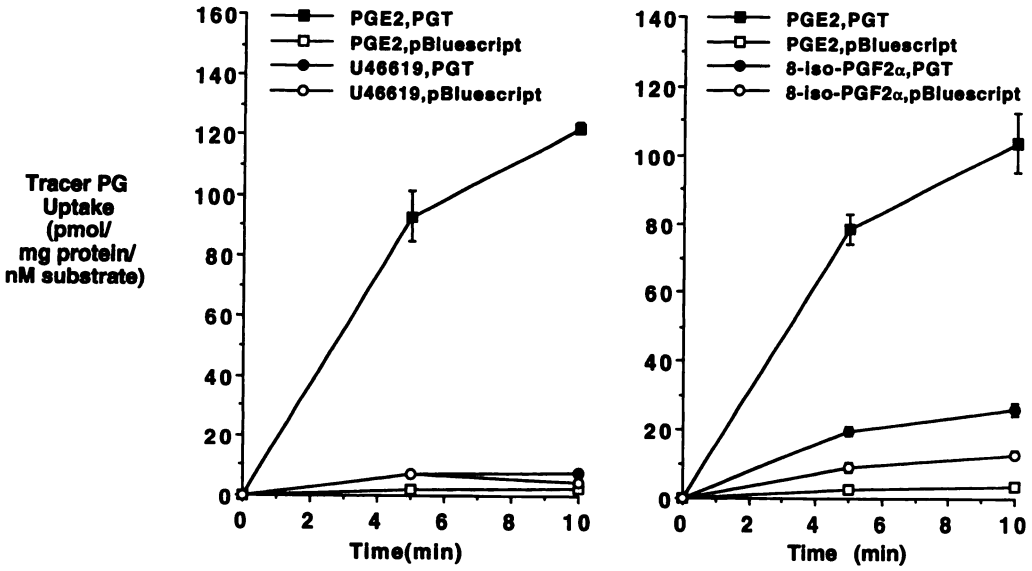


Fig. 3. Uptake of [^{3}H]PGE_2, [^{3}H]U46619, or [^{3}H]8-iso-$PGF_{2\alpha}$ by rat PGT expressed in HeLa cells. In each case, HeLa cell monolayers were transfected with either the full-length PGT cDNA PGT or with a vector control pBluescript. Results are normalized for an uptake medium substrate concentration of 1 nM. *Left*, [^{3}H]PGE_2 versus [^{3}H]U46619. *Right*, [^{3}H]PGE_2 versus [^{3}H]8-iso-$PGF_{2\alpha}$. Two monolayers for each data point. Values are mean ± standard error. For some data points, the error bars are smaller than the symbol.

Discussion

The current study permits several broad conclusions to be made about the structural requirements of potential PGT substrates. Relative to that of PGE_2, a strong interaction (binding) between prostanoids and the transporter requires a 1-position COO^- group, a 15-position —OH group in the (*S*) configuration, and C13═C14. The affinity of PGE_2 for PGT is weakened by removal of the C5═C6, by β stereochemistry at C8, or by introduction of a bulky group from approximately C16 on. Reduction at C9 and/or C11 or formation of C9—C11 epoxides increases affinity for PGT. Finally, isoprostanes interact with PGT, and 8-iso-$PGF_{2\alpha}$ is transported.

The current approach rests on the ability of unlabeled prostanoids to block the PGT-mediated transport of tracer PGE_2. Although shared transported substrates must, by definition, compete with each other, it does not necessarily follow that every inhibitor is actually transported. Data from our initial report on PGT and from the current study indicate that there is, in general, a good concordance between the K_i values and the transport rates for most prostanoids, including PGE_1, PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2, 6-keto-$PGF_{1\alpha}$, iloprost, and 8-iso-$PGF_{2\alpha}$ (Ref. 10; Table 1 and Fig. 3).

In marked contrast, however, the prostanoid U46619, although a fairly effective inhibitor of PGT (K_i = 39 nM), was not itself transported (Fig. 3). This result is similar to recent data from our laboratory obtained with the oatp; we found that corticosterone inhibits oatp-mediated tracer bromosulfophthalein transport but is not transported (19). A similar dichotomy between the strength of an inhibitor and its ability to be transported in the kidney has also been reported for other organic anions (20). Thus, the list of substrates in Table 1, although ranked in order of inhibitor constants, does not necessarily represent a rank order of transport rates.

The high affinity of PGT for PGH_2 relative to PGE_2 is of particular interest. The active site of cyclo-oxygenase resides within the lumen of the ER (21, 22). We do not know whether PGT is particularly concentrated in, or targeted to, the ER, but we surmise that newly synthesized PGT, like other integral membrane proteins, almost certainly traverses this compartment *en route* to the plasma membrane. Because PGT at the plasma membrane readily permits PGs to pass from the extracytoplasmic to the cytoplasmic compartment, if PGT is present in the ER membrane, it would serve as a potential mechanism by which newly synthesized PGH_2 could traverse the ER membrane (i.e., from lumen to cytosol). Ongoing experiments, aimed at ultrastructural localization of PGT, should shed light on this hypothesis. We should also note that despite the care taken to prevent degradation of PGH_2 to PGE_2, it is possible that some conversion did occur. The difference in the K_i values obtained with PGH_2 (38.2 nM) versus PGE_2 (48.7 nM) suggests that not all of the former was converted into the latter. Nevertheless, the K_i value for PGH_2 must be considered an upper limit.

Despite the inability to use inhibition profiles to completely predict transport, we can use the current approach to generate testable hypotheses regarding prostanoid binding sites within PGT. Our present model of the transporter, based on hydropathy analysis and on the location of conserved residues of rat and human PGT and oatp, suggests that three charged residues reside within each of three putative membrane spans. In the case of rat PGT, these are Glu78 in span 2, Arg560 in span 11, and Lys613 in span 12. A long term goal is to understand how these three residues interact, if at all, with full or partial charges on the surface of prostanoid molecules.

In this context, electrostatic potential surface modeling of three selected substrates (Fig. 2) yields the following insights. In the first example, reduction of the C9 ketone of PGE_2 to the C9 hydroxyl of $PGF_{2\alpha}$ changes a surface electroneutral region to an electropositive one (Fig. 2, *middle*, *arrows*), but this has no effect on the affinity for, or transport by, PGT (10).

In a second example, PGE_2, which has a region of electroneutrality on the cyclopentane ring, has a 3.8-fold lower affinity than 11-deoxy-PGE_2, in which this region is mildly electropositive (Fig. 2, *top*, *arrows*). The relative weakness of this 11-position change in potential suggests that this region of the substrate molecule may bind at some distance from charged residues in the transport pathway.

In the third example, a striking difference in affinity is apparent when PGE_2, which has a C15—OH in the (*S*) configuration, is compared with either 15(*R*)-PGE_2 (24-fold de-

crease in affinity) or 15-keto-PGE_2 (>100-fold decrease in affinity) (Table 1). In Fig. 2 (*bottom*), electrostatic potential surface models of these three molecules are viewed from a common perspective. The arrow tip might be considered to indicate the position of a hypothetical carboxyl group within the substrate pathway, perhaps arising from residue Glu78. Such modeling would predict strong electrostatic attraction ("binding") to the region of electropositivity shown in red on 15-(*S*)-PGE_2, weaker binding between Glu78 and the same region of 15-(*R*)PGE_2, and no attraction between Glu78 and the same region (now electroneutral) of 15-keto-PGE_2. We are currently testing this hypothesis directly by combining site-directed mutagenesis with substrate binding affinity analysis.

The transport of 8-iso-$PGF_{2\alpha}$ by PGT demonstrated here has important potential ramifications. Isoprostanes are stereoisomers in which the side chains are oriented *cis* in relation to the cyclopentane ring, as opposed to cyclo-oxygenase-derived PGs in which the side chains are exclusively oriented *trans* (23). Isoprostanes are formed, for the most part, by free radical-catalyzed peroxidation of arachidonic acid independent of the cyclo-oxygenase enzyme (12, 24). The F_2 isoprostanes have been implicated as markers of oxidative injury *in vivo*, and 8-iso-$PGF_{2\alpha}$ has vasoconstrictive activity (12). Because free F_2 isoprostanes and their metabolites are found in plasma and in urine in significant concentrations (12, 25), they presumably undergo clearance and metabolism, yet little is known about these pathways. Taken with the high likelihood that PGT represents a major plasma membrane permeability step in the clearance of $PGF_{2\alpha}$ (see below), we hypothesize that PGT may also mediate this step in the clearance of one or more of the isoprostanes. Further studies are required to address this important issue.

Finally, our results compare quite favorably with those on prostanoid clearance obtained by Eling *et al.* (4–6, 26) using the isolated perfused rat lung preparation. In studies examining the ability of several prostanoids to inhibit the uptake of tracer PGE_1, the rank order of inhibitory capacity was $PGE_2 > PGE_1 > PGD_2 >$ 13,14-dihydro-$PGE_{2\alpha} > PGD_1 \approx PGF_{2\alpha}$ methyl ester $\gg$ 15(*R*)-PGE_2 (6). Our sequence is only slightly different: $PGE_2 > PGD_2 \approx PGE_1 > PGD_1 >$ 13,14-dihydro-$PGE_{2\alpha} >$ 15(*R*)-$PGE_2 > PGF_{2\alpha}$ isopropyl ester (Table 1). Our rank order also compares favorably with the more limited study of Robinson and Hoult (27).

On the other hand, Eling *et al.* (6, 26) reported that tracer PGA_1 and PGB_1 were not transported by the perfused rat lung, results that seem to be inconsistent with the current K_i values of these prostanoids against PGT (Table 1). However, when these same investigators used lung perfusion conditions comparable to the uptake solutions used here (i.e., a Ringers-type buffer with no albumin), PGA_1 and PGB_1 were both taken up by rat lung at approximately two thirds the rate of PGE_1 (26). Although our results with unlabeled PGB_1 are quite consistent with these results (K_i = ~72 nM), our higher K_i value for PGA_1 (286 nM) seems more consistent with the absence of pulmonary uptake that Eling *et al.* (6, 26) obtained with albumin-containing perfusates. Further *in vitro* studies using the cloned rat PGT and albumin-containing uptake solutions, as well as direct testing of tracer PGA_1 transport by PGT, may clarify this discrepancy.

Given that PGT mRNA is strongly expressed in the lung, especially in the rat (10), the striking concordance between our results for the cloned rat PGT transporter and those obtained using the isolated rat lung strongly suggests that PGT represents the major, if not the only, route for carrier-mediated uptake of at least some PGs across the plasma membrane of pulmonary cells.

In summary, the rat cDNA PGT, expressed transiently *in vitro*, demonstrates substrate selectivity, consistent with a role in the clearance and metabolism of PGFs and PGFs. Because PGT also transports the isoprostane 8-iso-$PGF_{2\alpha}$, this transporter may play a role in the metabolism of these compounds.

Acknowledgments

We thank Dr. Carey K. Bagdassarian for the electrostatic potential surface calculations.

References

1. Ferreira, S. H., and J. R. Vane. Prostaglandins: their disappearance from and release into the circulation. *Nature (Lond.)* **216**:868–873 (1967).
2. McGiff, J. C., N. A. Terragno, J. C. Strand, J. B. Lee, A. J. Lonigro, and K. K. Ng. Selective passage of prostaglandins across the lung. *Nature (Lond.)* **223**:742–745 (1969).
3. Piper, P. J., J. R. Vane, and J. H. Wyllie. Inactivation of prostaglandins by the lungs. *Nature (Lond.)* **225**:600–604 (1970).
4. Eling, T. E., and M. W. Anderson. Studies on the biosynthesis, metabolism and transport of prostaglandins by the lung. *Agents Actions* **6**:543–546 (1976).
5. Anderson, M. W., and T. E. Eling. Prostaglandin removal and metabolism by isolated perfused rat lung. *Prostaglandins* **11**:645–677 (1976).
6. Eling, T. E., H. J. Hawkins, and M. W. Anderson. Structural requirements for, and the effects of chemicals on, the rat pulmonary inactivation of prostaglandins. *Prostaglandins* **14**:51–60 (1977).
7. Moncada, S., R. Korbut, S. Bunting, and J. R. Vane. Prostacyclin is a circulating hormone. *Nature (Lond.)* **273**:767–768 (1978).
8. Dusting, G. J., S. Moncada, and J. R. Vane. Recirculation of prostacyclin (PGI2) in the dog. *Br. J. Pharmacol.* **64**:315–320 (1978).
9. Nakano, J., E. Angaard, and B. Samuelsson. 15-Hydroxy-prostaglandin dehydrogenase: prostaglandins as substrates and inhibitors. *J. Biochem.* **11**:386–389 (1969).
10. Kanai, N., R. Lu, J. Satriano, Y. Bao, A. W. Wolkoff, and V. L. Schuster. Identification and characterization of a prostaglandin transporter. *Science (Washington D. C.)* **268**:866–869 (1995).
11. Lu, R., N. Kanai, Y. Bao, and V. L. Schuster. Cloning, tissue expression, and functional characterization of a human prostaglandin transporter (Abstract). *Kidney Int.* **6**:757 (1995).
12. Morrow, J. D., and L. J. Roberts II. The isoprostanes: current knowledge and directions for future research. *Biochem. Pharmacol.* **51**:1–9 (1995).
13. Fuerst, T. R., E. G. Niles, F. W. Studier, and B. Moss. Eukaryotic transient expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA* **83**:8122–8126 (1986).
14. Blakely, R. D., J. A. Clark, G. Rudnick, and S. G. Amara. Vaccinia-T7 RNA polymerase expression system: evaluation for the expression cloning of plasma membrane transporters. *Anal. Biochem.* **194**:302–308 (1991).
15. Chan, B., N. Kanai, J. Satriano, R. Lu, Y. Bao, and V. L. Schuster. The prostaglandin transporter PGT is an anion exchanger (Abstract). *Kidney Int.* **6**:753 (1995).
16. Frisch, M. J., G. W. Trucks, M. Head-Gordon, P. M. W. Gill, M. V. Wong, J. B. Foresman, B. G. Johnson, H. B. Schlegel, M. A. Rolb, E. S. Replogle, R. Gomperts, J. L. Andres, K. Raghavachari, J. S. Binkley, C. Gonzalez, R. L. Martin, D. J. Fox, D. J. DeFrees, J. Baker, J. J. P. Stewart, and J. A. Pople. *Gaussian 92: Revision A.* Gaussian, Pittsburgh (1992).
17. Politzer, P., and D. G. Truhlar, eds. *Chemical Applications of Atomic and Molecular Electrostatic Potentials*. Plenum Press, New York (1981).
18. Satriano, J., N. Kanai, R. Lu, Y. Bao, and V. L. Schuster. "OATP-2": a novel prostaglandin transporter: identification of -SH groups near the substrate binding site (Abstract). *J. Am. Soc. Nephrol.* **5**:320 (1994).
19. Kanai, N., R. Lu, Y. Bao, A. W. Wolkoff, and V. L. Schuster. Transient expression of the organic anion transporter "oatp" in mammalian cells: identification of candidate substrates. *Am. J. Physiol.* **270**:F319–F325 (1996).
20. Essig, A., and J. V. Taggart. Competitive inhibition of renal transport of *p*-aminohippurate by other mono-substituted hippurates. *Am. J. Physiol.* **199**:509–512 (1960).
21. Otto, J. C., D. L. DeWitt, and W. L. Smith. N-Glycosylation of prostaglandin endoperoxide synthases-1 and -2 and their orientations in the endoplasmic reticulum. *J. Biol. Chem.* **268**:18234–18242 (1993).
22. Picot, D., P. J. Loll, and R. M. Garavito. The X-ray crystal structure of the

membrane protein prostaglandin H2 synthase-1. *Nature (Lond.)* **367**:243–249 (1994).
23. Morrow, J. D., J. A. Awad, H. J. Boss, I. A. Blair, and L. J. Roberts II. Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed *in situ* on phospholipids. *Proc. Natl. Acad. Sci. USA* **89**:10721–10725 (1995).
24. Pratico, D., J. A. Lawson, and G. A. Fitzgerald. Cyclooxygenase-dependent formation of the isoprostane, 8-epi prostaglandin F2α. *J. Biol. Chem.* **270**:9800–9808 (1995).
25. Morrow, J. D., B. Frei, A. W. Longmire, J. M. Gaziano, S. M. Lynch, Y. Shyr, W. E. Strauss, J. A. Oates, and L. J. Roberts II. Increase in circulating products of lipid peroxidation (F2 isoprostanes) in smokers: smoking as a cause of oxidative injury. *N. Engl. J. Med.* **332**:1198–1203 (1995).
26. Hawkins, H. J., A. G. Wilson, M. W. Anderson, and T. E. Eling. Uptake and metabolism of prostaglandins by isolated perfused lung: species comparisons and the role of plasma protein binding. *Prostaglandins* **14**:251–259 (1977).
27. Robinson, C., and J. R. S. Hoult. Inactivation of prostaglandins in the perfused rat lung. *Biochem. Pharmacol.* **31**:633–638 (1982).

Send reprint requests to: Dr. Victor L. Schuster, Ullmann 617, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: schuster@aecom.yu.edu