Identification of lactate as a driving force for prostanoid transport by prostaglandin transporter PGT

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Chan, Brenda S., Shinichi Endo, Naoki Kanai, and Victor L. Schuster. Identification of lactate as a driving force for prostanoid transport by prostaglandin transporter PGT. Am J Physiol Renal Physiol 282: F1097–F1102, 2002; 10.1152/ajprenal.00151.2001.—We previously characterized the prostaglandin (PG) transporter PGT as an exchanger in which \( ^{3} \text{H} \)PGE\(_{2}\) influx is coupled to the efflux of a countersubstrate. Here, we cultured HeLa cells that stably expressed human PGT under conditions known to favor glycolysis (glucose as a carbon source) or oxidative phosphorylation (glutamine as a carbon source) and studied the effect on PGT-mediated \( ^{3} \text{H} \)PGE\(_{2}\) influx. PGT-expressing cells grown in glutamine exhibited a 2- to 4-fold increase in \( ^{3} \text{H} \)PGE\(_{2}\) influx compared with the antisense control, whereas cells grown in glucose exhibited a 14-fold increase. In the presence of 10 vs. 25 mM glucose during the uptake, there was a dose-dependent increment in \( ^{3} \text{H} \)PGE\(_{2}\) influx. \( \text{Cis} \) inhibition of \( ^{3} \text{H} \)PGE\(_{2}\) influx occurred with lactate at physiological concentrations (apparent \( K_{\text{m}} \) = 48 ± 12 mM). Preloading with lactate caused a dose-dependent \( \text{trans} \) stimulation of PGT-mediated \( ^{3} \text{H} \)PGE\(_{2}\) uptake, and external lactate caused \( \text{trans} \) stimulation of PGT-mediated \( ^{3} \text{H} \)PGE\(_{2}\) release. Together, these data are consistent with PGT-mediated PG-lactate exchange. Cells engaged in glycolysis would then be poised energetically for prostanoid uptake by means of PGT.

PROSTAGLANDINS (PGS) AND THROMBOXANES have widespread physiological and pathophysiological effects on nearly all cellular processes, including, but not limited to, cardiovascular, gastrointestinal, respiratory, reproductive, renal, and immune systems (56). Because of their broad biological effects, PGS modulate their activity in an autocrine fashion, i.e., they are synthesized by intracellular enzymes at or near their sites of action, exit the cell by simple diffusion (13), and are presented to adjacent PG receptors. Thereafter, extracellular PGS must be metabolized in situ within seconds before they are able to reach the general circulation (17, 21). This loss of biological activity is accomplished through cellular uptake followed by intracellular oxidation (1, 17, 43, 49).

Our laboratory previously identified the novel, broadly expressed PG transporter PGT, whose substrates include PGE\(_{2}\), PGF\(_{2\alpha}\), PGD\(_{2}\), and thromboxane-B\(_{2}\) (24). Understanding the molecular mechanism and driving force of PG transport by PGT is necessary to identify its role in PG homeostasis for the organism. PG transport by PGT is Na\(^{+}\), Cl\(^{-}\), and H\(^{+}\) independent and appears to occur by obligate anion exchange (13, 24).

Interestingly, in previous studies from our laboratory, concentrative PG uptake was reduced by glycolysis inhibitors and varied with cellular ATP production (13). There is known coupling between glycolysis and PG metabolism. Inhibition of renal papillary glycolysis by various maneuvers increases net PG release, whereas increasing the supply of glucose decreases PG release (22, 57). Our studies showed that PG transport by PGT exhibited a time-dependent overshoot that resulted in transient accumulation of \( ^{3} \text{H} \)PGE\(_{2}\) (13), which is consistent with exchange in which \( ^{3} \text{H} \)PGE\(_{2}\) influx is coupled to the efflux of a countersubstrate that exhibits a falling outwardly directed gradient. Taken together, the data suggested that PGT-mediated influx of PGS may be coupled to the efflux of an intracellular metabolic end product involved in the generation of ATP by glycolysis.

In the present study, we cultured HeLa cells that stably express PGT under conditions known to favor glycolysis or oxidative phosphorylation and studied the effect on PGT-mediated transport. Our data indicate that the direction and magnitude of PG transport by means of PGT vary directly with the transmembrane concentration gradient of lactate, which is consistent with PG-lactate exchange.

MATERIALS AND METHODS

Materials

Glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, glycerolaldehyde-3-phosphate, 1,3-diphosphoglycerate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, pyruvate, and lactate were purchased from Sigma and were the purest grade available. \( ^{3} \text{H} \)PGE\(_{2}\) was from DuPont-New England Nuclear.

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Stable Transfection of PGT in HeLa Cells

HeLa cells (ATCC) were grown in Dulbecco's modified Eagle's medium plus 5% fetal bovine serum and 100 U/ml penicillin-streptomycin (GIBCO). Full-length sense and antisense PGT cDNAs were cloned into the vector pMEP4 (Invitrogen). HeLa cells were transfected with Lipofectin (GIBCO) and pMEP4-antisense human PGT or pMEP4-sense human PGT and cultured in selective media containing hygromycin (600 μg/ml; GIBCO) 48 h later. After 14–18 days, resistant colonies were selected and expanded in selective media.

Cell Culture

Stable transfectants were maintained in humidified incubators with 5% CO₂ at 37°C in Dulbecco's modified Eagle's medium plus 5% fetal bovine serum and 100 U/ml penicillin-streptomycin. In experiments analyzing the effects of glycolysis on PGT-mediated transport, stable transfectants were grown overnight as monolayers on 35-mm dishes in Dulbecco's modified Eagle's medium with 2 mM glutamine, glucose plus 25 mM lactate, or glucose plus 25 mM 2-deoxyglucose supplemented with 5% fetal bovine serum.

Transport Assays

Influx measurements. The cell monolayers were washed twice with a balanced salt solution (BSS; in mM) 135 NaCl, 13 H-HEPES, 13 Na-HEPES, 2.5 CaCl₂, 1.2 MgCl₂, 0.8 MgSO₄, and 5 KCl. Influx measurements were initiated by the addition of [³H]PGE₂ to the flux media (BSS). Influx measurements were carried out at room temperature over 10 min and were terminated by aspiration of the incubation media followed by two rapid washings with ice-cold 5% BSA in BSS and two additional washings with ice-cold BSS. Cells were scraped into 1 ml saline, mixed with a liquid scintillation cocktail (National Diagnostics), and analyzed by liquid scintillation counting. Influx values were calculated as the percentage ± SE of the total [³H]PGE₂ released at 2-, 5-, and 10-min intervals.

RESULTS

Our laboratory previously reported that the PG transporter PGT is probably an obligatory, electrogenic anion exchanger (13). Moreover, our laboratory demonstrated that transport is reduced by inhibiting oxidative phosphorylation and glycolysis, suggesting that the countercurrent(s) may be a product(s) of intermediary metabolism (13). On the basis of these observations, we cultured HeLa cells that stably expressed human PGT under conditions known to favor glycolysis or oxidative phosphorylation, and then we studied the effect on PGT-mediated [³H]PGE₂ influx.

HeLa cells stably transfected with pMEP4-antisense human PGT showed virtually no uptake of [³H]PGE₂, which is consistent with previous data from our laboratory (13, 33, 45) and others (4, 8) that PGs at physiological pH enter cells poorly using simple diffusion. In contrast, HeLa cells transfected with pMEP4-sense human PGT showed a 27-fold increase in [³H]PGE₂ uptake over that in antisense control (data not shown). All subsequent experiments were performed with the stably expressing sense PGT HeLa cells.

It has been well established that cells in culture can utilize either glutamine or glucose to provide cell energy, depending on the availability of the respective substrates (9, 30, 46, 62). To test the hypothesis that PGT-mediated transport is dependent on metabolism, we examined the degree to which transport is dependent on oxidative phosphorylation or glycolysis by incubating our stable transfectants overnight in media containing 2 mM lactate with or without 25 mM glucose. Changing the pattern of metabolism had no effect on cell morphology; however, the cell-doubling times were ~33 h with glutamine and 24 h with glutamine plus glucose. No significant difference in the intracellular ATP concentrations were noted (data not shown), which is consistent with reports in the literature (46). However, there was a marked difference in the ability of PGT-expressing PGT cells to accumulate...
[\textsuperscript{3}H]PGE\textsubscript{2} as a function of glycolysis. As shown in Fig. 1, PGT-expressing cells grown in glutamine plus 25 mM glucose showed a 14-fold increase in [\textsuperscript{3}H]PGE\textsubscript{2} influx compared with PGT-expressing cells grown in glutamine without glucose or in glutamine with 25 mM 2-deoxyglucose as indicated. Values are means ± SE of 3 experiments.

To test intermediary metabolites as candidate substrates, we performed cis-inhibition experiments by adding metabolites to the outside of the cell during [\textsuperscript{3}H]PGE\textsubscript{2} influx. External substrates other than PGE\textsubscript{2} would be expected to reorient the exchanger toward the cytoplasm, rendering it unavailable to bind and internalize external tracer PGE\textsubscript{2}. As shown in Table 1, several glycolytic products inhibited [\textsuperscript{3}H]PGE\textsubscript{2} influx;

\textbf{Table 1. Cis-inhibition of [\textsuperscript{3}H]PGE\textsubscript{2} uptake by glycolytic end products}

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>K\textsubscript{i}, mM</th>
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<tbody>
<tr>
<td>Glucose-6-phosphate</td>
<td>31 ± 12</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Fructose-1,6-diphosphate</td>
<td>51 ± 11</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate</td>
<td>15 ± 11</td>
</tr>
<tr>
<td>1,3-Diphosphoglycerate</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>3-Phosphoglycerate</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2-Phosphoglycerate</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>26 ± 12</td>
</tr>
<tr>
<td>Lactate</td>
<td>48 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3–6 preparations of duplicate dishes for each inhibition constant (K\textsubscript{i}) value.

however, only lactate inhibited [\textsuperscript{3}H]PGE\textsubscript{2} influx at a concentration that might be approached in the cytoplasm. In accordance with this concept, in cells grown in glutamine overnight, the lactate concentration of the external media (which is likely in equilibrium with the cytoplasm at that point) was 3.5 ± 0.5 vs. 29 ± 6.1 mM in cells grown in glutamine plus glucose. This sevenfold increase in lactate concentration compares favorably with the sixfold increase in [\textsuperscript{3}H]PGE\textsubscript{2} uptake in cells grown in the presence of glucose. In contrast, medium pyruvate concentrations were similar in the two groups (119 ± 13 vs. 123 ± 18 \textmu M). We cannot exclude the possibility that under nonglycolytic conditions, metabolites other than the lactate shown in Table 1 might serve as substrates for PGT.

If lactate serves as the countersubstrate for PGE\textsubscript{2} on the PGT anion exchanger, then the rate of lactate efflux should vary with the rate of PGE\textsubscript{2} accumulation. As demonstrated in Fig. 3, net [\textsuperscript{3}H]PGE\textsubscript{2} uptake decreased pari passu with the rate of lactate release over time, suggesting that PGT-mediated transport is dependent on intracellular lactate. This is consistent with coupling between [\textsuperscript{3}H]PGE\textsubscript{2} uptake and lactate efflux by means of PGT.

If lactate is transported by PGT, it should accelerate PGE\textsubscript{2} transport, i.e., cytoplasmic lactate would be expected to reorient the exchanger toward the extracellular space, rendering it more available to bind

Fig. 1. Effect of culture media on prostaglandin transporter PGT-mediated [\textsuperscript{3}H]PGE\textsubscript{2} uptake. HeLa cells stably transfected with sense PGT were grown overnight in media containing 2 mM glutamine with or without 25 mM glucose or 2 mM glutamine with 25 mM 2-deoxyglucose as indicated. Values are means ± SE of 3 experiments.

Fig. 2. Effect of glucose concentration on PGT-mediated [\textsuperscript{3}H]PGE\textsubscript{2} uptake. pMEP-PGT stable transfectants were exposed to varying concentrations of glucose during 10-min transport assays. Values are percent increase ± SE of 3 experiments compared with 0 glucose.

Fig. 3. Relationship of time-dependent PGT-mediated net [\textsuperscript{3}H]PGE\textsubscript{2} accumulation and lactate efflux. Ability of PGT-expressing cells to accumulate [\textsuperscript{3}H]PGE\textsubscript{2} decreases as lactate efflux decreases over time. Values are means ± SE of 2 experiments.
and internalize external tracer PGE₂. To test this, we incubated the stable transfectants overnight in glutamine media so that the intracellular lactate concentrations before the experiment were low. Then, we incubated the cells in BSS in the absence or presence of 10 or 25 mM lactate for 15 min before the [³H]PGE₂ transport assay. As shown in Fig. 4, this produced a dose-dependent increase in [³H]PGE₂ uptake, i.e., lactate transstimulated [³H]PGE₂ uptake.

The converse experiments were also performed. Stable transfectants were loaded with [³H]PGE₂, and the rate of [³H]PGE₂ efflux was measured in the absence and presence of extracellular lactate. As shown in Table 2, trans lactate caused a significant increase in [³H]PGE₂ efflux, consistent with PGT-mediated lactate-PGE₂ anion exchange.

DISCUSSION

We previously found that PGT-mediated PG uptake varied directly with intracellular ATP metabolism (13). However, as PGT demonstrates no ATP binding motifs and has no homology to the P-type ATPases, we concluded that ATP is probably indirectly involved in PG transport. We therefore hypothesized that production of an intracellular countersubstrate coupled to PG uptake may be dependent on cellular metabolism. In the present study, we established HeLa cells that stably expressed human PGT and studied the effect of altering glucose metabolism on PGT-mediated [³H]PGE₂ uptake. We favored oxidative phosphorylation or glycolysis by supplying cells with glutamine or glutamine plus glucose, respectively, in an overnight incubation. We found that the presence or absence of glucose in the culture media does not result in significant changes in ATP levels. These results are in accordance with those of Reitzer et al. (46), who also found no change in intracellular citric acid intermediates with these maneuvers. Nonetheless, this maneuver results in extremely variable levels of glycolytic intermediates (46). Our data clearly indicate that substantial PG influx could not be supported in the absence of glycolysis. On the other hand, acute exposure of cells to glucose for only 10 min during the transport assay caused a dose-dependent increase in PGT-mediated PG uptake, which is consistent with the coupling of transport to glucose metabolism.

Several results presented here support intracellular lactate as the driving force for prostanoid uptake by means of PGT. First, the degree of increase in lactate concentration in cells cultured in glucose was similar to the degree of increase in PG uptake. Second, the time-dependent net accumulation of [³H]PGE₂ varied directly with the rate of lactate efflux. Third, preloading cells with lactate resulted in a dose-dependent augmentation of subsequent [³H]PGE₂ influx. Taken together, these data provide strong evidence that intracellular lactate drives PGT-mediated PG uptake.

We hypothesize that lactate is coupled to PG uptake by means of lactate-PG exchange. Several glycolytic metabolites cis inhibited PG influx; however, the Kₘ for lactate is closer to known cytoplasmic lactate levels than those for the other metabolites (7, 19, 34, 48, 52, 54). Indeed, the medium lactate concentration of cells cultured in glucose was 29 ± 8.5 vs. 3.5 ± 0.7 mM, respectively. On the assumption that the cytoplasmic and medium lactate concentrations are at equilibrium after overnight incubation, these data mean that the lactate concentration within cells was well within the range to serve as a substrate for PGT. In contrast, pyruvate was present at low levels in cells cultured with and without glucose [medium (pyruvate) = 119 vs. 123 μM, respectively], which argued against it being a substrate. Further evidence for lactate-PG exchange is the trans acceleration of [³H]PGE₂ efflux by the presence of external lactate; i.e., an inwardly directed lactate gradient results in lactate influx in exchange for PG efflux.

Lactate production under aerobic conditions has been described in many tissues, including lung (28, 32, 40, 47), kidney (38, 60), brain (36, 42), vascular smooth muscle (5, 35, 41), heart (2, 20), and skeletal muscle (11, 23). The presence of outwardly directed lactate gradients in diverse cell types suggests that PGT is poised for PG uptake rather than for release in vivo. Accordingly, using a number of in vitro expression systems, we have failed to demonstrate augmented PG release from cells expressing PGT; on the other hand,

![Diagram](http://ajprenal.physiology.org/)

**Fig. 4.** Effect of lactate preincubation on subsequent PGT-mediated net [³H]PGE₂ uptake. Stable transfectants grown overnight in glutamine media were preincubated with balanced salt solution (BSS) or BSS plus 10 or 25 mM lactate for 15 min before [³H]PGE₂ transport assays. Values are percent increase ± SE of 5 experiments compared with 0 lactate.

<table>
<thead>
<tr>
<th>Efflux Media</th>
<th>2 min</th>
<th>5 min</th>
<th>10 min</th>
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<tr>
<td>0 mM</td>
<td>15 ± 0.9</td>
<td>29 ± 0.9</td>
<td>48 ± 3.1</td>
</tr>
<tr>
<td>25 mM lactate</td>
<td>20 ± 0.7*</td>
<td>40 ± 1.4*</td>
<td>66 ± 3.4*</td>
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</table>

Values are means ± SE (n = 3). PGT-expressing cells were preloaded with [³H]PGE₂, washed, and incubated in balanced salt solution in the presence and absence of 25 mM lactate. [³H]PGE₂ efflux is expressed as the percentage of [³H]PGE₂ released during 2, 5, and 10 min of incubation. *P < 0.05, significantly different from cells incubated in balanced salt solution, using Student’s t-test.
we observed that expression of PGT at the plasma membrane results in less net PG release compared with controls (Chan BS, Bao Y, and Schuster VL, unpublished observations).

Taken together, these results suggest that PGT may be involved in reuptake of newly synthesized PGs. This model is similar to that of synaptic signaling, in which plasma membrane neurotransmitter transporters regulate extracellular neurotransmitter levels by uptake. Both prostanoids and neurotransmitters are synthesized and released at their sites of action, where they activate cell surface receptors. In the case of neurotransmission, termination of signaling is accomplished by local reuptake by high-affinity transporters (25, 26, 61). Here, we propose that net release of PGs is controlled by PGT-mediated reuptake within or near cells that release PGs. In concordance with this hypothesis, recent data from our laboratory have localized PGT to cells known to synthesize and release PGs (3).

A reuptake model can explain two previously puzzling papers that showed that inhibiting glycolysis in renal papillae increases the rate of PGE\textsubscript{2} release. Herman et al. (22) found that incubation of renal papillae with 2-deoxyglucose or the glycolytic inhibitor NaF increased net PGE\textsubscript{2} release. Similarly, Tannenbaum et al. (57) found that increasing amounts of glucose in the buffer suppressed net PGE\textsubscript{2} release. One explanation for these findings is that glycolysis promotes PG reuptake by PGT, resulting in a reduction in net PG release.

This hypothesis has ramifications for control of circulation and has potential for explaining vasomotor control during tissue ischemia. Occlusion of blood flow causes release of PGs and PG-mediated vasodilatation (6, 10, 15, 58). In our model, an increase in extracellular lactate in ischemic tissue would create an adverse gradient for PGT-mediated PG reuptake into cells such that the net release of PGs would be augmented.

In addition, Kawamura et al. (27) showed that the antiapoptotic effect of PGE in PC-12 cells requires PG uptake by means of PGT. This raises the possibility that extracellular PGs are taken up by cells to signal cell proliferation. In the case of some malignancies, cell proliferation involves abrogation of apoptosis (14, 39, 44, 51, 59). Tumor cells generate large amounts of lactic acid under aerobic conditions (18, 29, 55). Tumor cells generate large amounts of lactate in ischemic tissue would create an adverse gradient for PGT-mediated PG reuptake into cells such that the net release of PGs would be augmented.

In summary, our data demonstrate that PGT-mediated uptake of PGs is dependent on glycolysis and that transport is coupled to lactate by a mechanism consistent with lactate-PG exchange. The present model supports a role for PGT in the uptake of PGs in vivo and provides a molecular explanation for events in which metabolic derangement affects PG release and activity.

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REFERENCES


4. Baroody RA and Bito LZ. The impermeability of the basic cell membrane to thromboxane-B\textsubscript{2} prostacyclin and 6-keto-PGF\textsubscript{1a}. Prostaglandins 21: 133–142, 1981.


