Intracellular redox status affects transplasma membrane electron transport in pulmonary arterial endothelial cells

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Several endothelial cell TPMET systems have been identified on the basis of different electron acceptor and/or donor specificities (6, 10, 30, 31, 37, 44, 45, 47). At least one such system can utilize the cell membrane-impermeant thiazine electron acceptor, toluidine blue O polyacrylamide (TBOP), as an electron acceptor (3, 10). Given that thiazines are acceptors for a fairly wide array of oxidoreductase systems and that the molecular size of the TBOP polymer precludes cell entry in either the oxidized or reduced forms, TBOP is a particularly useful probe for studying cellular mechanisms involved in TPMET (3, 10). The objective of the present study was to examine the influence of intracellular redox status on pulmonary arterial endothelial cell TBOP reduction via TPMET.

The pyridine nucleotide redox poise is probably the most direct measure of cellular redox status. In the present study, we attempted to manipulate the cytoplasmic redox status of pulmonary arterial endothelial cells and to determine the effects on both intracellular pyridine nucleotides and thiazine reductase TPMET activity. Intracellular NADH, NAD+, NADPH, and NADP+ were quantified by KOH extraction of the cells, followed by high-performance liquid chromatography (HPLC). TPMET was measured using an assay for TBOP reduction. The results indicate that this endothelial cell TPMET system is, in fact, sensitive to cellular redox status. The more highly consistent correlation of NADH/NAD+ than NADPH/NADP+ is consistent with NADH as a likely electron donor for this TPMET system.

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METHODS

Materials. Potassium hexacyanoferrate (III) [K₃Fe(CN)₆], ferricyanide, 2-deoxy-D-glucose (2-DG), iodoacetate (IOA), HEPES, lactate dehydrogenase (LDH) assay kit 340-LD, ATP, and Hoechst-33258 were purchased from Sigma Chemical (St. Louis, MO). Trypsin, penicillin-streptomycin, and RPMI 1640 tissue culture medium were from GIBCO (Grand Island, NY). Fetal bovine serum was from Hyclone Laboratories (Logan, UT), and Biosilon beads were from Nunc (Roskilde, Denmark). Type CLS2 collagenase was obtained from Worthington Biochemical (Freehold, NJ), and diiododocarbocyanine-acetylated low-density lipoprotein was purchased from Bio-Rad Laboratories (Stoughton, MA). Protein determinations were performed using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA). NAD⁺, NADP⁺, NADPH, and NADH were purchased from Boehringer Mannheim (Indianapolis, IN).

Endothelial cell culture. The bovine pulmonary arterial endothelial cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 30 mg/ml 1-glutamine from segments of calf pulmonary artery obtained from a local meat processing plant as previously described (30). The cells were identified as endothelial cells by cobblestone morphology, which was observed by phase-contrast microscopy and accumulation of diiododocarbocyanine-acetylated low-density lipoprotein, as observed by fluorescence microscopy.

To prepare microcarrier beads coated with cells for experiments carried out in spectrophotometric cuvettes, cells between passages 4 and 20 that had grown to confluence in T-75 tissue culture flasks were detached from the flasks by treatment with trypsin (0.05% wt/vol) in Hanks’ balanced salt solution (HBSS) containing 5.5 mM glucose. Approximately 5 × 10⁶ cells were seeded onto 3 g (dry wt) of gelatin-coated (2% vol/wt) Biosilon beads (mean diameter 230 μm). The cells on the beads were cultured in biological stirrers (Techne, Princeton, NJ) in the medium described above. Stirring (60 rpm) was intermittent (2 min on, 30 min off) during cell attachment (~6 h) and continuous thereafter. The cells were grown to confluence on the beads as determined by observation with phase-contrast microscopy.

Inhibitor treatments. For the inhibitor treatments, experimental and control conditions were carried out using cells from the same culture flask to control for cell culture variables, such as passage number. Inhibitor treatments were carried out in the same manner for studies of TPMET activity and for HPLC measurements of pyridine nucleotides, except as noted. Approximately 0.4- or 0.2-ml packed volume of cell-coated beads were aliquoted from the stirred culture flasks into 10 × 10 × 10-mm polystyrene spectrophotometric cuvettes (Sarstedt, Newton, NC) or conical-bottomed tubes for TPMET activity or HPLC studies, respectively. After the cell-coated beads settled, they were washed four consecutive times by resuspension in 3 ml of HBSS containing 10 mM HEPES, pH 7.4 (HBSS/HEPES), allowing the beads to settle between each wash. The cell-coated beads were resuspended in 3 ml of HBSS/HEPES containing the following treatments: none (control); 0.4 mM IOA; 0.4 mM IOA and 5 mM lactate; glucose-free HBSS/HEPES containing 10 mM 2-DG; or 2-DG and 5 mM lactate. The cells were incubated in the treatment media by mixing on a Nutator mixer at 37°C for 30 min before measurements of TPMET activity or pyridine nucleotide and ATP concentrations.

TBOP polymer. To prepare the TBOP polymer, toluidine blue O was incorporated in an acrylamide polymer by copolymerization of toluidine blue O methacrylamide and acrylamide as previously described (10). Polymer chains of <3.5 kDa were removed by dialysis through a 3.5-kDa cutoff membrane. The amount of reducible toluidine blue O per unit mass of the toluidine blue O polyacrylamide (TBOP⁺) was ~17 nmol/mg.

TPMET activity. TBOP⁺ reduction by the cells was measured in two ways. First, TBOP⁺ (2 mg/ml of HBSS/HEPES) was added to the cell-coated beads in spectrophotometric cuvettes, as previously described (10). Once the cell-coated beads had settled to the bottom of the cuvettes (~25 s), the initial absorbance of the medium above the settled beads was recorded at 590 nm with a Beckman DU 7400 spectrophotometer. The cuvette contents were then gently mixed with the Nutator mixer, and after 2.5-min intervals, the cuvettes were placed in the spectrophotometer where the cell-coated beads were allowed to settle. Absorbance was measured again, and the cuvettes were returned to the mixer.

The second means of measurement of TBOP⁺ reduction involved the use of the secondary electron acceptor, ferricyanide, to simplify the assay. The ferricyanide was used to reduce TBOP⁺ (TBOPH), which is otherwise subject to autooxidation. In contrast to TBOPH, the reduced form of ferricyanide, ferrocyanide, does not autooxidize. The assay was carried out as previously described (30) by washing the cell-coated beads and adding 3 ml of fresh HBSS/HEPES containing TBOP⁺ and ferricyanide (0.2 mg/ml and 600 μM, respectively) to the cells in cuvettes. The wash solutions and the assay mixture also contained the same inhibitor treatments to which the cells were exposed during the previous 30-min treatment incubations. The absorbance of the medium surrounding the cells was measured, as described above, at 421 nm. Ferrocyanide, TBOP⁺, and TBOPH do not absorb at these wavelengths, allowing the concentration of ferricyanide to be determined using an extinction coefficient of 1 mM⁻¹·cm⁻¹. TPMET activity is expressed as the rate of TBOP⁺ reduction, which was calculated as 0.5 times the rate of ferricyanide disappearance from the medium.

The total ferrocyanide plus ferricyanide in the medium at the conclusion of the experiments was determined by addition of ferric chloride and Ferene S [3-(2-pyridyl)-5,6-bis(2-[5-furylsulfonic acid]-1,2,4-triazine, disodium salt trihydrate; Aldrich, Milwaukee, WI]. The calculated ferrocyanide plus ferrocyanide recovered in the cell medium was 103.3 ± 1.5 (% mean ± SE), indicating that the disappearance of ferrocyanide from the medium was due to reduction of ferrocyanide and not to cell uptake of either the oxidized and/or reduced forms.

Pyridine nucleotide HPLC. To determine the effects of the treatments on intracellular pyridine nucleotides and ATP, an HPLC method adapted from Stocchi et al. (39) was used. The cells on the Biosilon beads (200-μl packed bead volume) were treated as described in Inhibitor treatments. After 30 min of treatment, the cells on the beads were allowed to settle to the bottom of the tubes. The medium was removed from the tubes with a fine-tipped micropipette tip to remove as much of the residual extracellular medium as possible, and 0.3 ml of ice-cold 0.5 N KOH and 0.1 ml of HBSS/HEPES were added simultaneously to the cells on the microcarrier beads. The samples were mixed vigorously for 10 s with a vortex mixer, placed in an ice bath for 3 min, mixed vigorously after 1.5 min, followed by the addition of ice-cold water (0.225 ml). After being mixed vigorously, the contents of the tubes were centrifuged for 1 min at 2,100 g at 4°C. The cell extract was removed from the beads, which were dried and weighed to determine the cell culture surface area. The pH of 0.4 ml of the supernatant was adjusted to ~6.0 by the addition of 10 μl
of 6 N HCl and 20 μl of 1 M ammonium acetate (pH 4.7). The pH was measured, and, if necessary, additional ammonium acetate solution was added to obtain a pH as close as possible to 6.0. After centrifugation at 2,100 g for 1 min at 4°C, the cell extract supernatant was filtered through a 0.22-μm cellulose acetate syringe filter (μStar syringe filters; Costar, Corning, NY), and 100 μl of the filtrate was injected into the HPLC system.

The HPLC system consisted of an autosampler fitted with a 100-μl sample loop, a dual piston pump, a variable wavelength detector, and a helium degasser (Hewlett Packard 1050, models 79865A, 79852A, 79853C, and 79856A, respectively). A Supelcosil octadecylsilane LC-18-T (3-μm particle size; 150 × 4.6 mm) column (Supelco, Bellefonte, PA) was used with a guard column (Upchurch, Oak Harbor, WA) that was packed with a pellicular medium composed of octadecyl groups chemically bonded to 37- to 53-μm glass beads (Whatman, Clifton, NJ).

The components of the cell extract were separated at room temperature at a flow rate of 1.0 ml/min using a mobile phase consisting of two solutions, solutions A and B, that were made with reagent grade H2O (18.0 MΩ cm resistivity) obtained from a Quad Style Nanopure water system (Barnstead/Thermolyne, Dubuque, IA). Both mobile phase solutions were continuously sparged with helium. Solution A was 64.35 mM potassium phosphate buffer (pH 6.0) containing 2.57 mM tetrabutylammonium hydrogen sulfate. Solution B was the same as solution A, except that it also contained 35.65% methanol (HPLC grade; Burdick and Jackson, Muskegon, MI). The mobile phase gradient for the separation was as follows: 2.5 min in solution A; a 2.5-min transition to 7:3 solution A:solution B; a 5-min transition to 1:1 solution A:solution B; a 10-min transition to 3.5:6.5 solution A:solution B; 3 min in constant 3.5:6.5 solution A:solution B; followed by a 4-min transition back to solution A. The column was reequilibrated in solution A for 11 min between separations.

The chromatograms were recorded on an integrator (Hewlett Packard 3396), and the data were transferred to a MATLAB program that allowed for peak area quantification against standard curves. Figure 1 is an example HPLC chromatogram showing intracellular KOH-extractable NADH, NAD+, NADP+, NADPH, and ATP. Peaks obtained from the cell extract chromatogram that correspond to the elution times of authentic standards of pyridine nucleotides and ATP are indicated with arrows. The elution times for authentic NAD+, NADP+, NADH, ATP, and NADPH were 8.2 ± 0.0, 11.7 ± 0.02, 15.8 ± 0.05, 16.8 ± 0.05, and 19.3 ± 0.06 min (means ± SE), respectively, for 13 injections.

Standard curves were generated by subjecting the standards to the same extraction procedure as the cells. The percent recovery of standards (means ± SE) throughout the extraction and HPLC procedure compared with standards prepared in water and injected directly onto the HPLC system was 86.3 ± 1.2, 91.8 ± 1.6, 95.8 ± 3.9, 87.7 ± 1.5, and 82.7 ± 0.7% (means ± SE, n = 3) for NAD+, NADH, NADP+, NADPH, and ATP, respectively. To further establish that the procedure resulted in accurate quantification of the KOH-extractable cell pyridine nucleotides and ATP, in five experiments, 0.1 ml of HBSS/HEPES containing a mixture of authentic NAD+, NADH, NADP+, NADPH, and ATP was added to the cells on the beads simultaneously with the KOH extraction medium in place of the 0.1 HBSS/...
ANOVA was followed by Tukey test (H11021P). The presence of 2-DG and lactate (10 and 5 mM, respectively).

The period was 4.5 the total cell LDH released into the medium during the study when the cells were lysed, and LDH activity in the extracellular medium was determined after each TBOP-absence of cells. The extracellular medium was removed from the cells, subjected to the extraction and HPLC procedures in the presence of 2-deoxy-D-glucose (2-DG, 10 mM) or TBOP in the presence of IOA and lactate (0.4 and 5 mM, respectively).

Table 1. Pyridine nucleotide and ATP concentrations in cells

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>NADH</th>
<th>NAD+</th>
<th>NADPH</th>
<th>NADP+</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pmol/cm² cell surface area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>5.6±0.3</td>
<td>77.5±4.3</td>
<td>9.8±0.6</td>
<td>1.7±0.1</td>
<td>724±40</td>
</tr>
<tr>
<td>IOA</td>
<td>5</td>
<td>1.9±0.3*</td>
<td>76.3±6.4</td>
<td>9.2±1.6</td>
<td>1.8±0.2</td>
<td>368±101*</td>
</tr>
<tr>
<td>2-DG</td>
<td>5</td>
<td>2.3±0.2*</td>
<td>62.1±5.9</td>
<td>7.4±0.6</td>
<td>2.2±0.1</td>
<td>440±39*</td>
</tr>
<tr>
<td>IOA + lactate</td>
<td>5</td>
<td>8.1±1.0*</td>
<td>71.4±3.9</td>
<td>10.9±0.9</td>
<td>1.6±0.1</td>
<td>670±50</td>
</tr>
<tr>
<td>2-DG + lactate</td>
<td>5</td>
<td>8.8±1.0*</td>
<td>59.2±5.1</td>
<td>7.8±0.7</td>
<td>1.5±0.1</td>
<td>491±41*</td>
</tr>
</tbody>
</table>

Pyridine nucleotide and ATP concentrations expressed as pmol/cm² cell surface area in control cells and cells exposed to glycolytic inhibitor treatments in the absence or presence of lactate, measured by HPLC. Values are means ± SE. *Significantly different from control; one-way ANOVA was followed by Tukey test (P < 0.05). IOA, iodoacetate; 2-DG, 2-deoxy-D-glucose.

RESULT

The reduction of TBOP⁺ to TBOPH by the bovine pulmonary arterial endothelial cells is shown in Fig. 2. When TBOP⁺ was added to the medium surrounding the cell-coated beads, the concentration of the polymer-oxidized dye moieties in the medium decreased until a steady state was reached (Fig. 2). The steady state represents the opposing rates of reduction by the cells and autooxidation within the medium (10). When the cells were removed from the medium (at the time represented by the dotted line in Fig. 2), the medium TBOP⁺ concentration returned to its initial value as the result of autooxidation unopposed by reduction by the cells. The complete recovery of TBOP⁺ in the medium upon reoxidation confirmed that the disappearance of TBOP⁺ was due to reduction by the cells.

Figure 3 shows that ferricyanide reduction by the cells depended on the presence of TBOP in the assay medium. There was almost no reduction of ferricyanide by the cells on this time scale in the absence of TBOP. The reduction of ferricyanide in the presence of cells and TBOP indicates reduction of TBOP⁺ to TBOPH by the cells. In the presence of TBOP, the ferricyanide reduction was zero order until the ferricyanide was essentially exhausted, as expected of a secondary electron acceptor that does not affect the primary reaction rate. Thus the rate of ferricyanide disappearance was used to calculate the TBOP⁻ reduction rate, as indicated in METHODS.

Figure 4 shows examples of the effects of the IOA and 2-DG treatments, respectively, on the rate of TBOP⁺ reduction by the cells. Both inhibitors decreased the rate of TBOP⁺ reduction (Fig. 4). When lactate was included in the medium along with either IOA or 2-DG, reduction was not inhibited (Fig. 4). The rate of TBOP⁺ reduction in the absence and presence of lactate but without either 2-DG or IOA was 215 ± 5 and 230 ± 10 pmol·min⁻¹·cm⁻², respectively, indicating that lactate alone did not substantially influence the rate of TBOP⁺ reduction by the cells in the absence of the inhibitors.
The pyridine nucleotide and ATP concentrations in control cells and cells exposed to IOA and 2-DG in the absence or presence of lactate are shown in Table 1. NADH concentrations were lower in extracts of cells treated with IOA or 2-DG than in control cells. This effect of the inhibitors was not observed when lactate was included in the medium along with the inhibitors. No significant differences were detected among the mean values of NADH$^+/\text{NADH}^-$, NADPH$^+/\text{NADPH}^-$, and NADPH$^+/\text{NADP}^+$ concentrations under control and inhibitor treatment conditions without or with lactate. ATP concentrations were lower in cells treated with IOA or 2-DG than in control cells. Lactate prevented the effect of IOA, but not of 2-DG, on ATP concentrations.

The effects of the treatment conditions on the TBOP$^+$ reduction rate and intracellular NADH/NAD$^+$ and NADPH/NADP$^+$ ratios are shown in Figs. 5 and 6. In general, changes in reduction rate and the poise of the NADH/NAD$^+$ and NADPH/NADP$^+$ pairs were in the same direction. However, the correlation was greater for the NADH/NAD$^+$ ratio than for the NADPH/NADP$^+$ ratio, which can be appreciated by the representation of the data in Fig. 7.

**DISCUSSION**

The results suggest that the pulmonary arterial endothelial cell surface thiazine reductase is sensitive to the cytoplasmic redox status as reflected in the ratios of oxidized/reduced pyridine nucleotides. Thus it would appear that within the range of ratios achieved in this study, the intracellular supply of reducing equivalents is an important factor controlling the rate of electron transport to the extracellular electron acceptor.

The concept behind the manipulations carried out is that both IOA and 2-DG result in inhibition of the cytosolic source of NADH, but at two different sites along the glycolytic pathway, glyceraldehyde-3-phosphate dehydrogenase and hexokinase, respectively (18). Inhibition at either site can be overridden by the addition of lactate as an alternative source of NADH via the lactate dehydrogenase reaction. These manipulations carried out is that both IOA and 2-DG result in inhibition of the cytosolic source of NADH, but at two different sites along the glycolytic pathway, glyceraldehyde-3-phosphate dehydrogenase and hexokinase, respectively (18). Inhibition at either site can be overridden by the addition of lactate as an alternative source of NADH via the lactate dehydrogenase reaction.
ulations have been used to examine the role of glycolysis in TPMET in endothelial and other cell types, as well as in other endothelial cell functions (7, 14, 25, 28, 32, 46, 48). However, their effects on endothelial intracellular NAD\(^+\), NADH, NADP\(^+\), NADPH, and ATP concentrations have not been previously determined.

In fact, there is little information available on endothelial cell pyridine nucleotide concentrations in general. In the one study available for comparison in which human umbilical vein endothelial cell NAD\(^+\), NADP\(^+\), NADH, NADPH, and ATP were all quantified, the concentrations were 78.4 ± 4.3, undetectable, 9.5 ± 1.5, 7.6 ± 1.1, and 595 ± 13 pmol\(\mu\)g\(^{-1}\)DNA (means ± SE, n = 18), respectively, compared with 45.6 ± 2.5, 0.98 ± 0.1, 3.29 ± 0.2, 5.8 ± 0.3, and 427 ± 23 pmol\(\mu\)g\(^{-1}\)DNA (means ± SE), respectively, in the present study (2). Considering differences in cell source, culture conditions, etc., the baseline levels of intracellular pyridine nucleotides and ATP in this study were reasonably close to those measured in the human umbilical vein endothelial cells. The comparison implies some consistency in related metabolic functions of the two endothelial cell types in their respective culture conditions and between the different extraction and HPLC techniques.

As is generally true with the use of inhibitors, unintended effects are potentially confounding. IOA, a sulfhydryl alkylating agent, is not a highly specific inhibitor. While 2-DG appears to be rather specific for the hexokinase reaction, hexokinase is also a source of glucose-6-phosphate for NADPH via the pentose phosphate pathway, which is consistent with the 2-DG effects on the NADPH/NADP\(^+\) ratio. However, the common effects observed using the two inhibitors and the reversal of the common effects by lactate suggest that it is their common effects on the glycolytic source of NADH that is most important for their effects on this TPMET activity. It also appears to confirm that neither inhibitor effect was a result of direct TPMET inhibition. This further distinguished this endothelial thiazole reductase from that of at least one other TPMET system, the NADH-ascorbate free radical reductase of rat liver cells, which is apparently directly inhibited by IOA (41).

The results are also consistent with NADH actually being an electron donor for this TPMET system, which has been the interpretation of the results with similar metabolic inhibitors in other TPMET systems. For example, TPMET reduction of the tetrazolium WST-1 in various cell lines, including Jurkat, HeLa, and isolated spleen cells, was inhibited by IOA and 2-DG and enhanced by cyanide (7, 8). These results were interpreted as indicating that TPMET reduction of WST-1 in these cells was dependent on glycolytic NADH production, with sparing of cytoplasmic NADH by cyanide. A somewhat similar pattern was observed in a neuroblastoma cell dichloroindolphenol acceptor TPMET system that was stimulated by glucose, inhibited by 2-DG, but unaffected by cyanide (48). The correlation between NADH levels and oxygen reduction by TPMET systems, in some cell types, has been considered to be a demonstration of TPMET in cellular NADH/NAD\(^+\) balance when glycolysis is the dominant cellular energy source of ATP (5, 7, 8, 23).

Although this study does not directly impute NADH as the direct electron donor to the thiazole TPMET system, it is not obvious what other intermediate electron donor would be likely to link NADH levels to this TPMET activity. Ascorbate has been ruled out (30), and NADPH rather than NADH is generally considered to be the cytoplasmic source of electrons for regenerating intracellular electron donors such as glutathione (36). One possible intermediate between NADH and the TPMET system might be coenzyme Q10, which is a plasma membrane constituent in at least some cell
types where it is apparently maintained in the hydroquinone form by a plasma membrane NADH-cytochrome b₅ reductase (1, 19, 42).

The greater correlation between NADH/NAD⁺ and TBOP reduction rate than between NADPH/NADP⁺ and TBOP reduction rate does not rule out a role for NADPH either as a direct electron donor or as member of a more complicated electron transfer chain. The experimental conditions targeted glycolysis. Thus the proportionate range achieved for NADPH concentrations was not as great as for NADH. It would be interesting to expand that range in future studies. However, metabolic inhibitors and other tools available for manipulating intracellular NADPH tend to provide a more complex spectrum of cellular effects. Thus a more complex protocol will probably have to be devised to further address this question.

The measurements made in the current study were of total cytosolic and mitochondrial KOH-extractable cellular pyridine nucleotides, with no distinction between protein-bound and protein-free forms. However, the correlation between the TBOP⁺ reduction rate and the cellular NADH or NADPH concentrations or reduced/oxidized pyridine nucleotide ratios in the presence of the glycolytic inhibitors is consistent with the supposition that the differences observed in total cell NADH or NADPH reflected differences in available cytosolic levels. The observation that the ratio of the NADH/NAD⁺ and NADPH/NAD⁺ pairs are so different, despite having virtually identical midpoint potentials, may be at least partly attributable to compartmentation and protein binding. However, this discrepancy has been seen even in the cytosolic pool, where it is apparently due in part to the reaction kinetics of several reversible, highly active cytosolic dehydrogenases and their substrate and product concentrations (43). Other factors involved in regulating the poise of the redox pairs include the rate of transfer of reducing equivalents between cellular compartments, the rate of utilization of NADH in the respiratory chain (43), and the rate of utilization of NADPH, predominately for regeneration of intracellular antioxidants and biosynthetic reactions.

The pulmonary endothelial thiazine reductase in the intact lung and in cells in culture reduces monomeric thiazine drugs to lipophilic cell-permeant compounds (9, 10). This physicochemical change dramatically influences the tissue distribution of this drug class (11, 15, 21, 24, 26). The results of the present study reveal that the redox status of the tissue, as reflected in the poise of the reduced/oxidized pyridine nucleotides, is likely to have an impact on the tissue distribution of thiazines as well as other compounds that are electron acceptors for this TPMET system. Because reduction commonly increases the lipophilicity and, therefore, the membrane permeation of such electron acceptors, their use in studying intracellular mechanisms involved in TPMET can be complicated by their own effects on intracellular metabolism (7, 29). Because TBOP cannot enter the cells in the oxidized or reduced forms, this complexity in interpretation is avoided.

In conclusion, these results indicate that cytoplasmic redox status, as reflected in the NADH/NAD⁺ and NADPH/NADP⁺ ratios, affects TPMET to thiazine compounds and also indicate that the NADH/NAD⁺ appears to be more directly correlated with TPMET activity. In addition, these studies suggest that future studies evaluating the possibility that TBOP⁺ reduction may provide a nondestructive index of changes in cell redox status may be worthwhile.

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