Lactate Transport in Freshly Isolated Human Fetal Retinal Pigment Epithelium

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Purpose. To study transport mechanisms for small monocarboxylic acids in the apical and basolateral membranes of freshly isolated, human fetal retinal pigment epithelium.

Methods. The epithelium was mounted in a small Ussing chamber that allowed separate perfusion of both the apical and basal compartments and simultaneous measurements of intracellular pH, transepithelial potential, and tissue resistance. Intracellular pH was measured using a pH-sensitive dye, 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein.

Results. When 10–100 mM lactate or pyruvate was added to the apical bath the cells acidified by 0.10–0.25 pH units. There were no differences between the initial rates of intracellular acidification produced by L-lactate and D-lactate. These rates could be described as Michaelis-Menten functions of the concentrations of lactate and pyruvate. The $K_m$ values were: 42 ± 12 mM for L-lactate and 34 ± 8 mM for pyruvate. The rates of acidification caused by 50 mM L-lactate were reversibly reduced by 44% or 35% after apical administration of probenecid (2 mM) or α-cyano-4-hydroxycinnamate (2 mM), and irreversibly reduced by 78% after apical administration of the sulfhydryl-reagent mersalyl acid (2 mM). The intracellular acidifications caused by apical pyruvate (50 mM) were completely and reversibly inhibited by 50 mM apical L-lactate. Addition of 50 to 100 mM lactate to the basal bath caused intracellular alkalinizations, which could be inhibited by Na+ removal in the basal bath or by 2 mM α-cyano-4-hydroxycinnamate in the apical bath.

Conclusion. These results suggest the presence of a proton–lactate cotransport system in the apical membrane and a Na+-dependent transport mechanism for the lactate anion in the basolateral membrane. Invest Ophthalmol Vis Sci. 1994;35:434-442

Lactic acid is the end product of anaerobic glycolysis. Both the neuroretina and the retinal pigment epithelium (RPE) produce lactate under aerobic conditions in vitro.1-3 In intact pigs in vivo, Törnquist and Alm measured higher lactate concentrations in mixed choroidal venous blood than in arterial blood.6 This suggests that the pig retina produces lactate in vivo.

Retina has a high lactate concentration. In rats the retina contains 7.2 mmol lactate per kilogram of tissue,7 whereas the brain contains only 1 to 2 mmol lactate per kilogram of tissue.8 In rat brain the retinal lactate concentration increases after ischemia; it may reach 15 mmol/kg tissue after 30 minutes of complete ischemia.8 The retina is close to hypoxia, even under physiologic conditions. In cats, the oxygen tension in the retinal outer nuclear layer is almost zero in dark-adapted retinas and only around 30 mm Hg in light-adapted retinas.9

Lactate transport systems have been found in the blood–retinal barrier and in the blood–brain barrier in rats.10,11 These transport systems have not yet been studied at the cellular level. The RPE is an important part of the blood–retinal barrier, because it is a single layer of epithelial cells in the outer retina that separates the photoreceptors from the choriocapillaries.

We studied the transport of lactate and pyruvate across the RPE membranes by monitoring intracellular pH (pH_i) during rapid changes in the extracellular concentrations of lactate or pyruvate. We identified an
H⁺, lactate cotransport system in the apical membrane, and an Na⁺-dependent transport mechanism for the lactate anion in the basolateral membrane.

MATERIALS AND METHODS

Tissues

The freshly isolated RPE-choroid preparation was obtained from human fetuses electively aborted at gestational ages between 19 and 23 weeks. The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained after the nature and possible consequences of the study were explained. Research protocols were approved by the University of California Committee for the Protection of Human Subjects. Immediately after the abortion the eyes were enucleated, placed in transport Ringer’s solution on ice and received at the laboratory within approximately 2 hours.

In the laboratory, the eyes were cut at the ora serrata and the anterior portion removed. The posterior part of the globe was cut in two sections. Each section was transferred to cold control Ringer’s solution, and the retina was gently peeled away from the RPE. A circular RPE-choroid sheet was then dissected away from the sclera and placed on a circular nylon mesh, 7 mm in diameter. Tissue and mesh were finally mounted into a modified Ussing chamber.

Chamber for Microscopic Observation

The modified Ussing chamber has been described elsewhere. It consists of an open basal chamber and a small, closed apical chamber. The apical chamber contained ≈ 7 µl of solution; it was fitted with a glass coverslip that allowed microscopic observation of the apical side of the epithelium. The apical and the basal chambers were fitted with agar-Ringer bridges for measurement of transepithelial potential and with Ag-AgCl electrodes for passage of current pulses and measurement of transtissue resistance. A temperature probe in the basal chamber monitored temperature of the solution in contact with the tissue at 37°C. The solutions used to perfuse the tissue were kept in a temperature-controlled box. By adjusting the temperature in the box, it was possible to hold the temperature of the solution in contact with the tissue at 37°C. Solution changes were made through a manifold at fixed height that allowed them to be done rapidly and free of pressure pulses. Before switching solutions at the manifold, they were allowed to run for approximately 20 seconds to flush out stagnant solutions in the tubing. This also avoided temperature pulses due to cooling and insufficient gassing. There was a delay of ≈ 25 seconds between the solution change pulses due to cooling and insufficient gassing. There was a delay of ≈ 25 seconds between the solution change at the manifold and the arrival of new solution in the chamber.

Solutions

Transport Ringer’s solution contained NaCl (116.4 mM), NaHCO₃ (26.2 mM), KCl (5 mM), MgCl₂ (1.8 mM), NaH₂PO₄ (1 mM), D-glucose (5.6 mM), and CaCl₂ (1.0 mM).

Control Ringer’s solution contained Na⁺ (126.5 mM), K⁺ (5 mM), Mg²⁺ (0.8 mM) Ca²⁺ (1.0 mM), Cl⁻ (123.6 mM), H₂PO₄⁻ (1 mM), HEPES (26.2 mM), and glucose (5.6 mM). In solutions containing L-lactate, D-lactate, pyruvate, or gluconate an equimolar amount of Cl⁻ was replaced. All solutions were titrated to pH 7.40. Just before the experiments glutathione (1 mM) was added to all solutions.

The transport Ringer’s solution was gassed with 10% O₂, 5% CO₂, and 85% N₂. All other solutions were gassed with 10% O₂ and 90% N₂. Oxygen was restricted to 10% because it has been shown that higher concentrations are toxic to bovine RPE in vitro. BCECF-AM was obtained from Molecular Probes, Inc. (Eugene, OR). Na-D-lactate was obtained from FLUKA Chemical Co. (Ronkonkoma, NY). All other reagents were obtained from Sigma Chemical Company (St. Louis, MO).

Measurements of pH

We measured pH using the pH-sensitive dye 2',7'-bis (carboxyethyl) - 5 (6) - carboxyfluorescein (BCECF) with a ratio fluorimetry technique. A 75 W xenon lamp (Osram, Berlin, Germany) was used as excitation light source. Every half-second the excitation wavelength was switched between 440 and 480 nm using interference filters with a 10 nm bandwidth (Omega Optical, Inc., Brattleboro, VT). The epifluorescent emission was measured between 520 and 560 nm using a photomultiplier tube (Thorn EMI Gencom, Fairfield, NJ). The voltage and fluorescence signals were digitized and stored on an IBM-AT-type microcomputer. The technique and computer software used for data acquisition and analysis were based on a design by Dr. Chester Regan (BioRad, Boston, MA). The RPE cells were loaded with BCECF by perfusing the apical chamber for 10 to 20 minutes with a solution containing ≈ 10 µM of the membrane-permeable, nonfluorescent acetomethyl derivative of BCECF. The acetomethyl groups were cleaved intracellularly (presumably by cytoplasmic esterases) to produce the membrane-impermeable fluorescent dye BCECF. After loading tissue with dye, the intracellular
fluorescence signal slowly decreased, yet stable intracellular pH measurements could readily be obtained for at least 2 hours after the tissue was loaded with BCECF. The background fluorescence of the RPE was measured before the epithelium was loaded with BCECF and was subtracted from the fluorescence measured after BCECF loading.

After an experiment was completed, a four-point calibration between pH 6.6 and pH 7.8 was made.17 Calibration solutions contained KCl (135.6 mM), Na-HEPES (10 mM), MgCl₂ (0.8 mM), D-glucose (5.6 mM), and CaCl₂ (1.8 mM). The K⁺/H⁺ exchanger nigericin (10 to 20 μM) was added to the apical calibration solutions to allow equilibration of internal and external pH.17

Unless otherwise specified all data are presented as mean ± SEM (n = number of experiments).

RESULTS

Steady-State Values

The resting, steady-state intracellular pH in the human fetal RPE cells was 7.03 ± 0.04 (n = 16). The average transepithelial potential and the transepithelial resistance were 0.86 ± 0.21 mV and 153 ± 25 Ωcm² (n = 16). All values were measured when the experiments began.

Monocarboxylate Transport Across the Apical (Retina Facing) Membrane

Lactate-Dependent Intracellular Acidification. When 10 to 100 mM lactate was administered into the apical bath for 1 minute, the cells acidified by 0.10–0.25 pH units (Table 1, Figure 1). The changes in pH were linear for at least the first 9 seconds after onset. In this phase the initial rates of intracellular acidification were compared in five tissues (Fig. 1). No significant differences were found between the rates of intracellular acidification and the intracellular pH recorded just before the addition of lactate are shown in Table 1.

In four control experiments 100 mM Cl⁻ was replaced with gluconate, and no intracellular acidifications were seen (Fig. 1). Thus, the intracellular acidifications were caused by addition of lactate and not by the simultaneous removal of Cl⁻.

Administration of apical lactate caused small increases in transepithelial potential, but no changes in tissue resistance (Figure 1). The transepithelial potential increases were also observed when apical Cl⁻ was replaced with gluconate. They may represent a combination of Cl⁻-diffusion potentials generated at the agar bridges and Cl⁻-shunt potentials generated in the paracellular pathways.18

L-lactate and D-lactate produced similar intracellular acidifications. The effects of L-lactate and D-lactate were compared in five tissues (Fig. 1). No significant differences were found between the rates of intracellular acidification caused by L-lactate and D-lactate in concentrations of 20 mM (n = 8), 50 mM (n = 8), and 100 mM (n = 10); paired t tests, P > 0.1.

TABLE 1. Summary of Results

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>L-Lactate</th>
<th>D-Lactate</th>
<th>Pyruvate</th>
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<td></td>
<td>−dpHᵢ/dt (min⁻¹)</td>
<td>pHᵢ</td>
<td>n</td>
</tr>
<tr>
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<td>0.32 ± 0.06</td>
<td>7.11 ± 0.04</td>
<td>6</td>
</tr>
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<td>20</td>
<td>0.45 ± 0.03</td>
<td>7.06 ± 0.03</td>
<td>11</td>
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<tr>
<td>30</td>
<td>0.58 ± 0.06</td>
<td>7.08 ± 0.04</td>
<td>6</td>
</tr>
<tr>
<td>40</td>
<td>0.65 ± 0.07</td>
<td>7.09 ± 0.04</td>
<td>6</td>
</tr>
<tr>
<td>50</td>
<td>0.75 ± 0.03</td>
<td>7.05 ± 0.03</td>
<td>33</td>
</tr>
<tr>
<td>75</td>
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<td>7.09 ± 0.04</td>
<td>6</td>
</tr>
<tr>
<td>100</td>
<td>0.98 ± 0.10</td>
<td>7.02 ± 0.04</td>
<td>13</td>
</tr>
</tbody>
</table>
Lactate Transport in Human Fetal RPE

Pyruvate-Dependent Intracellular Acidifications. Administration of apical pyruvate caused intracellular acidifications similar to those observed after the addition of apical lactate (Fig. 2). The initial rates of intracellular acidifications caused by 10 to 100 mM apical pyruvate are shown in Table 1.

The presence of 50 mM apical L-lactate completely and reversibly blocked the intracellular acidification caused by 50 mM apical pyruvate (n = 5, Fig. 2). In these experiments, the previous administration of lactate lowered pH_j by 0.22 ± 0.02 pH units (n = 5). It is unlikely that this change in pH_j was responsible for the observed inhibition of the pyruvate response. In the absence of lactate, pyruvate caused easily detectable intracellular acidifications when added at resting pH_jS ranging from 6.71 to 7.25.

Inhibitors of Lactate Transport Across the Apical Membrane. The effects of four inhibitors on the rates of intracellular acidification caused by 50 mM L-lactate were investigated (Table 2). The inhibitors were: α-cyano-4-hydroxycinnamate (CHC), lactic acid isobutylerster, probenecid, and mersalyl acid. Figures 3A and 3B show that CHC (2 mM) and probenecid (2 mM) inhibited the intracellular acidifications caused by 50 mM L-lactate. The effects of both inhibitors were fully reversible. Lactic acid isobutylerster only marginally inhibited the acidification caused by 50 mM L-lactate, and its effect was not fully reversible (Fig. 3C).

Figure 3D shows that transient administration of 2 mM mersalyl acid into the apical bath for 1 minute acidified the cells, and that the subsequent administration of 50 mM L-lactate did not cause the intracellular acidification seen under control conditions. Long-term exposure (>5 min) to mersalyl acid (2 mM) resulted in irreversible loss of transepithelial potential. Therefore, mersalyl acid (2 mM) was administered into the apical bath for only 1–2 minutes, and then removed. After the inhibitor was removed, the effects of 50 mM L-lactate (Fig. 3D) were investigated. Compared to the previous control experiment, the initial rate of acidification caused by 50 mM L-lactate was reduced by 78% after mersalyl exposure. The effects of mersalyl acid were not reversible. Two tissues were allowed to recover for ≈20 min after mersalyl acid exposure. After this period 50 mM L-lactate caused intracellular acidifications, the rates of which were 28% and 65% of their previous control values.

Lactate Transport Across the Basolateral (Choroid Facing) Membrane

When 50 or 100 mM L-lactate was administered into the basal bath for 2 minutes, the cells alkalinized (Discussion). This is opposite to the intracellular acidifications observed after apical lactate administration (Fig. 4). Because of diffusion through the choroidal connective tissue, the onset of the intracellular alkalinations was delayed by ≈30 seconds. For at least 50 seconds after onset, the change in the intracellular pH was linear with time. The rate of intracellular alkalization was determined by linear regression in this phase; after 50 mM basal L-lactate this rate was 0.07 ± 0.01 pH units · min⁻¹ (n = 20).

No changes in intracellular pH were observed in four control experiments in which 100 mM basal Cl⁻...
was replaced with gluconate (not shown). Thus, it was
the administration of basal lactate, and not the simulta-
neous removal of basal Cl⁻, that produced the intra-
cellular alkalinizations shown in Figure 4.

The alkalinization caused by basal lactate could be
inhibited by 2 mM apical CHC (Figure 5A). In six tis-
ues, the initial rate of alkalinization was reduced by \( \approx \) 40% to 0.04 ± 0.01 pH units \( \cdot \) min⁻¹. Removal of basal extracellular Na⁺ also inhibited the intracellular alka-
linizations caused by basal lactate (Fig. 5B). The initial
rate in the absence of basal Na was reduced by \( \approx \) 50%
to 0.033 ± 0.005 pH units \( \cdot \) min⁻¹ (\( n = 5 \)).

DISCUSSION

Lactate and pyruvate transport across cell membranes
are known to occur by the following mechanisms: (1)
nonionic diffusion of the undissociated acids, (2) co-
transport with protons, (3) cotransport with Na⁺, and

FIGURE 3. The effects of 50 mM apical L-lactate on pHᵢ, transepithelial potential, and transep-itheelial resistance in the absence and the presence of 2 mM apical α-cyano-4-hydroxycimma-
rate (CHC, panel A); 2 mM apical probenecid (panel B); and 2 mM apical and lactic-acid-isoor-
butylester (LAIE, panel C); and before and after a 2-minute apical administration of 2 mM
mersalyl acid (MA, panel D).

FIGURE 4. The effects on pHᵢ, transepithelial potential, and transepithelial resistance of administration of 50 mM L-lactate in the apical bath (apical lac) followed by addition of 50
mM L-lactate to the basal bath (basal lac).
Lactate Transport in Human Fetal RPE

(4) transport of the monocarboxylic acids as "back passengers" on the Cl/\text{HCO}_3\text{ }^\text{-} transport system.\textsuperscript{19} The monocarboxylate, proton cotransport system accepts both lactate and pyruvate\textsuperscript{19\textendash}21; for convenience we will refer to it as H\textsuperscript{+}/lactate\textsuperscript{-} cotransport.

Transport of Lactate and Pyruvate Across the Apical Membrane

Both nonionic diffusion and H\textsuperscript{+}/lactate\textsuperscript{-} cotransport directly influence pH\textsubscript{i}, as protons are transported across the cell membrane. The intracellular acidifications observed after apical administration of lactate or pyruvate can therefore be explained by these modes of monocarboxylate transport (Figs. 1, 2).

No attempts were made to inhibit or control cellular metabolism. It is possible that influx of lactate or pyruvate changed cellular metabolism and thereby caused secondary changes in pH\textsubscript{i}. However, such metabolic effects on pH\textsubscript{i} were most likely minor, because lactate influx via the apical membrane and lactate influx via the basolateral membrane had opposite effects on pH\textsubscript{i} (Fig. 4).

Unlike nonionic diffusion, carrier-mediated monocarboxylate transport should exhibit saturation kinetics, competitive inhibition between substrates, and sensitivity to known inhibitors. In some systems but not in others, carrier-mediated lactate transport shows stereospecificity for L-lactate over D-lactate.

Kinetics of Lactate- and Pyruvate-Induced Acidifications. Figure 6 shows the initial rates of intracellular acidifications plotted against the concentrations of L-lactate, D-lactate, and pyruvate. The rates of intracellular acidification approached saturation as we increased the concentrations of lactate and pyruvate toward 100 mM. If the acidifications were caused by nonionic diffusion of the monocarboxylic acids, the relationship between the concentrations and the rates of acidification should be linear.

The rates of intracellular acidification could be described as Michaelis-Menten functions of the concentrations of L-lactate, D-lactate, and pyruvate (Fig. 6). The K\textsubscript{m} values were 42 ± 12 mM for L-lactate, 57 ± 16 mM for D-lactate, and 34 ± 8 mM for pyruvate (estimates ± SD). These values are all within the range of affinity constants (1.7 to 73 mM) previously reported for this cotransport.\textsuperscript{22\textendash}23 The Hill coefficients for L-lactate, D-lactate and pyruvate were 0.94 ± 0.04, 1.01 ± 0.12, and 0.98 ± 0.07, respectively (estimates ± SD). These values are all close to one, consistent with the existence of only one binding site for lactate or pyruvate on the cotransporter.

The above kinetic analysis should be viewed with caution, however, because it does not consider contribution to cell pH changes from nonionic diffusion and changes in cell metabolism. Although saturability of the intracellular acidifications suggests that they were primarily caused by H\textsuperscript{+}/lactate\textsuperscript{-} cotransport, a contribution from nonionic diffusion cannot be excluded. K\textsubscript{m} may thus be overestimated. Pyruvate is 23 times more dissociated than lactate at physiologic pH
(pK_a(lactate) = 3.86, pK_a(pyruvate) = 2.49). Because the pyruvate and lactate oil-water partition coefficients are similar, lactate should be more readily transported by nonionic diffusion than pyruvate. This may contribute to the slightly higher degree of saturation seen with pyruvate than with lactate (Fig. 6). The metabolic effects on pH_i caused by lactate and pyruvate may also be different. The conversion from lactate to pyruvate, catalyzed by lactate dehydrogenase, liberates a cytoplasmic proton, the reverse reaction consumes one.

In the kinetic analysis it is assumed that the rates of change in pH_i are proportional to the rates of H^+ influx, and thus to the rates of H^+,lactate^- cotransport. This is true only if the intracellular buffering power remains constant in the pH_i range of interest. In the current study the resting, pre-experimental pH values were slightly different in the experiments with L-lactate (7.06 ± 0.01, n = 81), D-lactate (6.94 ± 0.02, n = 54), and pyruvate (6.90 ± 0.03, n = 33). In frog RPE the intracellular buffering power has been shown to depend on pH_i.13

**Competitive Inhibition Between Lactate and Pyruvate.** The intracellular acidifications caused by apical pyruvate could be completely and reversibly inhibited by equimolar amounts of apical lactate (Fig. 2). This indicates that lactate and pyruvate shared the same transport mechanism in the apical membrane. It also indicates that nonionic diffusion was of minor importance for the intracellular acidifications caused by apical pyruvate. In other systems H^+,lactate^- cotransport also shows competitive inhibition between lactate and pyruvate.20-22

**Effects of Inhibitors.** CHC is a widely used inhibitor of H^+,lactate^- cotransport.22-28 We found that the drug reversibly inhibited the rates of lactate-induced intracellular acidification by 44% (Fig. 3A). This is also the case in skeletal muscle, kidney proximal tubule, and corneal epithelium, where CHC reduced the rates of lactate-induced intracellular acidifications by between 39% and 76%.26-28

Probenecid reversibly inhibited H^+,lactate^- cotransport in the human fetal RPE cells (Fig. 3B). Probenecid has been shown to block lactate efflux from central nervous system cells in intact rats,26 but it is not well characterized as an inhibitor of H^+,lactate^- cotransport at the cellular level. Lactic acid isobutyester only weakly and irreversibly inhibited H^+,lactate^- cotransport in the human fetal RPE cells (Fig. 3C). This contrasts with the situation in rabbit corneal epithelium, where the drug reversibly inhibited 40% of lactate-induced intracellular acidifications.28

Mersalyl acid irreversibly inhibited H^+,lactate^- cotransport through the apical membrane (Fig. 3D). This is not surprising because mersalyl acid is a well-known SH-reactant and inhibitor of H^+,lactate^- cotransport.22,28,30

**Stereospecificity of H^+,Lactate^- Cotransport.** The effects of apical L-lactate and apical D-lactate were compared and it was found that the two isomers produced the same rates of intracellular acidification (Figs. 1, 6). This indicates that H^+,lactate^- cotransport in human fetal RPE is not markedly stereospecific. Previous studies have shown great variability in the degree of stereospecificity of this cotransport.20,26,31,32 In erythrocytes from mammals the stereospecificity of H^+,lactate^- cotransport is species dependent. The cotransport is highly stereospecific in erythrocytes from rodents but not in erythrocytes from oxen and sheep.33

**Lactate Transport Through the Basolateral Membrane**

When lactate was added to the basal bath, the cells alkalinized (Fig. 4). Apical CHC inhibition of the alkalinization (Fig. 5A) indicates that the alkalinization is mediated by the apical H^+,lactate^- cotransporter as protons are transported out of the cell with the lactate. The alkalinizations were also inhibitable by basal Na^+ removal (Fig. 5B), suggesting Na^+-dependent entry of the lactate anion through the basolateral membrane, possibly by Na^+,lactate^- cotransport. A similar mechanism has been described in proximal tubules of salamanders.30

**Physiologic Implications**

It is likely that the RPE transports lactate from the retina to the choroid under physiologic conditions in vivo. The neuroretina produces lactate in vitro.3-5 In vivo in intact pigs it has been shown that mixed choroidal venous blood contains more lactate than arterial blood.6 It therefore is likely that the pig retina produces lactate in vivo, and that this lactate is transported across the RPE into the choroid, where it is removed by the choriocapillary microcirculation. If it is assumed that H^+,lactate^- cotransport has a 1:1 stoichiometry,22,26 the rate of H^+,lactate^- cotransport (J_{H^+,lactate^-}) across the apical membrane in the human fetal RPE can be calculated:

\[ J_{H^+,lactate^-} = -\frac{\partial p_{H^+_i}}{\partial t} - \beta \cdot h \]  

where \( \partial p_{H^+_i}/\partial t \) is the initial rate of change in pH_i, \( \beta \) is the RPE intracellular buffering power,13,34 and h = 0.001 cm is the estimated volume-to-surface ratio for the epithelium. Intracellular buffering power has not been measured in human fetal RPE, but its value in the frog is 10 mM/(pH unit)^{-1} in the physiologic range.13,34 In a previous study using human fetal RPE, the magnitude of NH4^-induced alkalinizations were
Lactate Transport in Human Fetal RPE

qualitatively consistent with this estimate. In the mammalian retina the extracellular lactate concentration is \( \approx 7 \text{ mM} \), and the value of \( \text{dpH}/\text{dt} \) (\( \approx 0.2 \text{ pH units} \cdot \text{min}^{-1} \)) at this concentration is extrapolated from the data shown in Figure 6. These values can be used in equation 1 to calculate \( J_{\text{actalc}} = 2 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \). The lactate production in the rat retina in vitro amounts to approximately \( 0.1 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \). It appears that the H⁺,lactate⁻ cotransport system in the RPE apical membrane has the capacity required to remove the lactate that is produced in retina under physiologic conditions in vivo.

If lactate is absorbed across the RPE, an exit mechanism is required at the basolateral membrane. This membrane incorporates a Na⁺-dependent transport system for lactate (Fig. 5B), the nature of this transport system is not clear, and it is not known if it mediates lactate efflux or influx. Because basal Na⁺ removal only inhibited the lactate-induced alkalinization by \( \approx 50\% \) (Fig. 5B), it is likely that the basolateral membrane also incorporates Na⁺-independent lactate transport systems. One may speculate that lactate exit through the basolateral membrane can proceed via the Cl⁻/HCO₃⁻ exchanger that has been found in this membrane.

The current study represents a first step in understanding the mechanisms involved in RPE lactate transport. These mechanisms have been studied in human fetal RPE but not confirmed in adult human RPE because of the scarcity of human donor tissue. However, the presence of similar transporters in both adult frog and adult bovine RPE (manuscripts in preparation) suggest that they are not solely developmental mechanisms.

The H⁺,lactate⁻ cotransport system found in the apical membrane appears to have the capacity required to clear the retinal lactate production. The Na⁺-dependent lactate transport system found in the basolateral membrane may represent Na⁺:lactate cotransport, but its role in transepithelial lactate transport under physiologic conditions remains to be elucidated. More generally, it will be important to understand how the activity of these lactate transport mechanisms are coordinated to modulate retinal metabolism.

Key Words
monocarboxylate transport, H⁺, lactate cotransport, retinal metabolism, retinal ischemia

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References


