Is active glucose transport present in bovine ciliary body epithelium?

Chu Yan Chan,1,2 Jeremy A. Guggenheim,3 and Chi Ho To2

1School of Optometry and Vision Sciences, Cardiff University, Wales, United Kingdom; and 2Laboratory of Experimental Optometry, Department of Optometry and Radiography, The Hong Kong Polytechnic University, Hong Kong, Hong Kong Special Administrative Region

Submitted 2 February 2006; accepted in final form 2 October 2006

Chan CY, Guggenheim JA, To CH. Is active glucose transport present in bovine ciliary body epithelium? Am J Physiol Cell Physiol 292: C1087–C1093, 2007. First published October 4, 2006; doi:10.1152/ajpcell.00048.2006.—Hyperglycemia is a major risk factor for diabetic cataract formation. Effective regulation of glucose transport by the ciliary body epithelium (CBE) is pivotal to normal glycemic control in the anterior eye, which in turn affects the glucose level of the crystalline lens. The present study aimed to characterize the glucose transport mechanisms across the bovine blood-aqueous barrier (BAB) represented by the CBE. With an Ussing-type chamber, the glucose transport kinetics were measured and characterized in the presence and absence of various glucose transporter inhibitors. The saturation characteristics of the CBE to glucose were estimated from an Eadie–Hofstee plot. The mRNA expression of glucose transporters in specific regions of the bovine CBE was assessed using RT-PCR. The trans-CBE glucose flux was found to be sensitive to the glucose transporter inhibitors cytochalasin B, phloretin, and phlorizin. The transport system had a kinetic constant of 5.3 mM and a maximum velocity of 349.5 nmol·h−1·cm−2. Gene expression for GLUT1, GLUT3, GLUT4, GLUT5, and SGLT2 was observed in both the pars plana and pars plicata regions of the bovine CBE. This study demonstrates that glucose transport across the bovine CBE is primarily passive in nature. However, the novel findings of 1) the presence of a phlorizin-sensitive glucose flux and 2) gene expression for SGLT2 mean that a potential role for active glucose transport cannot be ruled out. The elucidation of the exact function of SGLT2 in the bovine CBE may shed important light on the glucose transport and physiology of the BAB and inform future studies of glycemic control in relation to diabetic cataract formation.

Ussing-type chamber; reverse transcriptase-polymerase chain reaction; sodium-dependent glucose transporter; phlorizin

A good control of plasma glycemic level is crucial to the health of diabetic patients, although it is not always easy to achieve. Because of the fluctuating glycemic level in the blood stream, diabetic patients are prone to a number of ocular complications, including diabetic retinopathy (DR) (11) and diabetic cataract (32). Although the complete mechanism of diabetic cataract formation is still unknown, it is clear that hyperglycemia is the key factor in causing cataract and other diabetic-related pathological changes (45, 53, 58).

Cataract is one of the leading diabetic complications that causes blindness in both juvenile and late-onset diabetic patients (32). Diabetic cataract accounts for nearly 20% of all cataract surgeries in Western countries (27). Clinically, surgical removal of the crystalline lens is the mainstay of treatment for all cataracts, including diabetic cataract. This surgical procedure is generally safe but not without complications. An ideal approach to protect patients from these surgical complications would be to lower the incidence of diabetic cataract altogether. Research has been devoted to the use of drugs that can modify the pathological pathways in diabetic cataract formation. These approaches include the use of novel glycation inhibitors (36), antioxidants (1, 34), and aldose-reductase inhibitors (56, 57). In addition to the use of agents to target complications due to hyperglycemia, another plausible strategy would be to control glucose entry into the aqueous humor so that the normal glycemia can be maintained within the eye. Such an approach might prevent the appearance of abnormally high concentrations of glucose in the aqueous humor and drastically reduce many of the related downstream diabetic pathologies.

The major barrier to plasma glucose entering the anterior chamber of the eye is the ciliary body epithelium (CBE), which lines the aqueous-facing aspect of the ciliary body. The aqueous humor is the major source of glucose supply for many avascular ocular tissues such as the cornea and crystalline lens (10, 16, 59). Its glucose concentration is known to be comparable and related to that of the blood plasma (5, 13). The CBE is an epithelial bilayer that consists of the pigmented epithelium (PE) and the nonpigmented epithelium (NPE). These two layers join together in an apex-to-apex fashion. The basolateral surface of the NPE faces the aqueous humor, whereas the PE faces the ciliary stromal blood supply. Since glucose is a polar molecule that cannot pass the CBE bilayer by diffusion, the presence of integral membrane transporter proteins (i.e., the glucose transporter) is necessary to assist its transmembrane movement (3, 4, 7).

In general, a carrier-facilitated or passive transport mechanism is found to be the predominant mode of glucose transport in ocular tissues, such as the retinal pigment epithelium (RPE) [bovine (14, 61, 63), frog (17, 46), chick (44), rat (21, 55, 66)], the blood-aqueous barrier (BAB) [rat (18)], the corneal endothelium [rabbit (26)], and the crystalline lens [rat (16)]. Active sodium-coupled glucose transport also has been reported in rabbit conjunctiva (30, 31) and sheep RPE (51). Although glucose transport characteristics have been widely studied in the retina and RPE (22, 66, 68), little information is available specifically regarding the CBE. In addition, although many studies have documented the pattern of GLUT and SGLT mRNA expressions in ocular tissues including cornea, lenses, RPE, and retina, the glucose transporter gene expressions in the CBE have not been reported previously. In the present study, we investigated the glucose transport mechanisms across the bovine CBE. Different inhibitors were used to reveal a more
Table 1. Primers used in the RT-PCR study

<table>
<thead>
<tr>
<th>Glucose Transporter</th>
<th>Primer Sequence 5’-3’</th>
<th>Tm, °C</th>
<th>TA, °C</th>
<th>Product Size, bp</th>
</tr>
</thead>
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<tr>
<td>GLUT1-UP</td>
<td>GACAGCCACTACAGCATCTCCTGTT</td>
<td>68.7</td>
<td>64</td>
<td>548</td>
</tr>
<tr>
<td>GLUT1-DN</td>
<td>GATGTGGATGATGGCTGGTT</td>
<td>68.8</td>
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<tr>
<td>GLUT2-UP</td>
<td>CAGCTTCCAAGCAATGGCCA</td>
<td>66.4</td>
<td>62</td>
<td>296</td>
</tr>
<tr>
<td>GLUT2-DN</td>
<td>CTGGAGCAGACATCTCCACA</td>
<td>65.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT3-UP</td>
<td>CCCCTGCTGCTGGTGCTGA</td>
<td>67.1</td>
<td>63</td>
<td>532</td>
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<td>GLUT4-UP</td>
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<td>63</td>
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<td>GLUT5-UP</td>
<td>GCTTACGTCTCCGTGCTTA</td>
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<td>59</td>
<td>495</td>
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<tr>
<td>GLUT5-DN</td>
<td>GATGAACTTTGAGAGGCACCAGT</td>
<td>64.5</td>
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<tr>
<td>SGLT1-UP</td>
<td>GGATGGTGGCTCTGTGGA</td>
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<td>59</td>
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<tr>
<td>SGLT1-DN</td>
<td>GTGTCGCAGAAATACTGCTG</td>
<td>63.7</td>
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<td>SGLT2-UP</td>
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<td>SGLT2-DN</td>
<td>ACTGATTGAGAAGCCGTACAGG</td>
<td>69.9</td>
<td></td>
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</tr>
</tbody>
</table>

Tm, melting temperature; TA, annealing temperature; UP, upstream primer; DN, downstream primer; bp, base pair.

complete picture of the glucose transport mechanism of the CBE. Saturation characteristics were estimated with an Eadie-Hofstee analysis, and RT-PCR was used to study the mRNA expression profile of glucose transporters in both the pars plana and the pars plicata regions of the CBE. Since glucose transport across the CBE would directly determine the glucose concentration of the aqueous humor, its characterization would be meaningful and might provide new insights toward achieving good ocular glycemic control.

MATERIALS AND METHODS

Materials. [14C]-3-O-methyl-D-glucose ([14C]MDG) was purchased from NEN Life Science Products (Boston, MA). [3H]-l-glucose ([3H]LG), phlorizin, phloridzin, cytochalasin B, ouabain, dimethyl sulfoxide, and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Tissue preparation and dissection. Fresh bovine eyes were obtained from a local abattoir immediately after death. They were transported to the laboratory within an hour post mortem in an ice box. The bovine CBE was isolated and mounted in a Ussing-type chamber according to the method of To et al. (62). Briefly, all connective tissues and extraocular muscles around the globe were removed with a razor blade. Two radial incisions were made through the limbus to the equator. The cornea was excised, and the CBE was carefully isolated anteriorly from the sclera and posteriorly from the vitreous. Finally, a sector of CBE was isolated at a point around 5 mm behind the pars plana into the retina. The preparation was then soaked in Ringer solution for 10 min before mounting.

Tissue preparation and mounting. Before mounting, silicone lubricant (Dow Corning) was applied to the silicon plates of the Ussing chamber to enhance sealing of the tissue in the chamber during the experiment (62). HEPES-buffered Ringer solution was perfused into the chambers (via the perfusate inlets) at a rate of 10 ml/h by a microprocessor-controlled syringe infusion pump (Cole-Parmer Instrument). The composition of the Ringer solution was (in mM) 113.0 NaCl, 4.6 KCl, 21.0 NaHCO3, 0.6 MgSO4, 7.5 D-glucose, 1.0 glutamate (D-aspartate). The composition of the Ringer solution was (in mM) 113.0 NaCl, 4.6 KCl, 21.0 NaHCO3, 0.6 MgSO4, 7.5 D-glucose, 1.0 glutamate (D-aspartate). The preparation was maintained at 35–37°C by a heating jacket surrounding the chambers. Two CBE tissues were excised from the same bovine eye and mounted in two different chambers so that the unidirectional glucose fluxes in both directions (stroma to aqueous, Jsa; aqueous to stroma, Jsa) could be measured in tissues from the same eye simultaneously.

Electrical parameters. The transepithelial potential difference (PD) and the transepithelial resistance (Rr) were monitored using a Dual Voltage Clamp-1000 unit (World Precision Instruments, Sarasota, FL). Concurrently, a dual-channel flatbed chart recorder (BD-12E; Kipp & Aonen, Saskatoon, SK, Canada) was used to record the voltage and current output of the preparation.

Viability criteria. The CBE preparations were considered to be viable if they produced stable PD and Rr greater than −0.2 mV and 70 Ω·cm², respectively, during the first hour of mounting. They also were required to demonstrate a low diffusional LG flux (<70 nmol·h⁻¹·cm⁻², as suggested by To et al. (64)) and biphasic response to 0.1 mM ouabain addition to the aqueous side (64, 67). Preparations were rejected if they failed any one of these criteria.

Basal glucose flux measurement. After the tissue was stabilized for 60 min, the CBE preparation was short-circuited. HEPES-buffered Ringer solution containing radioactive [3H]LG and [14C]MDG was loaded to either the stromal or aqueous half chamber (the “hot” side). Concurrently, Ringer solution with no radioactive tracer was perfused to the opposite side (the “cold” side). After another 90 min, samples were collected from both half chambers. Thereafter, perfusates (2 ml) were collected at a 12-min interval. Each perfusate sample was mixed with 15 ml of biodegradable scintillation cocktail (NBCS104; Amersham Radiochemicals, Amersham, UK), and the radioactivity was measured with a liquid scintillation counter (1414 Winspectral DSA; Wallac, Helsinki, Finland). The count data allowed the calculation of unidirectional glucose fluxes (Jsa and Jsa) of the paired preparations.

The unidirectional flux (J) was calculated according to the following equation

\[ J = (C \cdot I \cdot V)/(H \cdot A \cdot T) \]

where C is cold side radioactivity (cpm), I is the concentration of the glucose in the Ringer solution (mM), V is the volume of perfusate collected in each interval (ml), H is the radioactivity (cpm) at the hot side, A is the cross-sectional area of the chamber cavity (cm²), and T is the collection time for each sample (h).

The unidirectional fluxes were calculated from the specific activity of the hot side and the rate of radioactive tracer appearance on the cold side with time. The flux value was expressed in nmol·h⁻¹·cm⁻².

Effects of inhibitors. To study the effects of different inhibitors, we perfused Ringer solution containing a particular inhibitor (0.1 mM phlorizin, 0.01 mM cytochalasin B, 0.1 mM phloridzin, or 0.1 mM ouabain) to both sides of freshly mounted preparations for 60 min. Thereafter, Ringer solution with both the radioactive tracers and the inhibitor was perfused to one side (i.e., the hot side), and Ringer solution containing only the inhibitor was perfused to the opposite side (i.e., the cold side). Perfusates were collected after another 90 min, and the radioactive fluxes were analyzed as described above.

Fig. 1. Typical effect of bilateral 0.1 mM ouabain addition (indicated by arrow) on transepithelial potential difference (PD) values of a bovine ciliary body epithelium (CBE) preparation.
Carefully removed using RNase-free scissors, and then the exposed ciliary processes were harvested using RNase-free forces, whereupon they were soaked in RNAlater, frozen, and stored at −80°C for later use.

The inner cortex of kidney was used as a positive control for this study. Pieces of kidney cortex tissue (~0.5 × 0.5 cm) were isolated with a sterile blade and treated in the same way as the CBE tissues.

The frozen tissues were powdered at liquid nitrogen temperature with a dismembrator (Mikro-Dismembrator U; B. Braun Biotech International) in the presence of TRI reagent (Sigma-Aldrich, Dorset, UK). RNA was obtained by phenol-chloroform extraction and repeated ethanol precipitation as described previously (23). RNA samples were treated with DNase (Qiagen, West Sussex, UK) according to the manufacturer’s instructions. All samples, including the kidney samples, were then repurified using an RNaseasy mini kit (Qiagen) to remove pigment (since melanin pigment from the PE of the CBE samples copurified with RNA). The final RNA sample was resuspended in 100 μl of RNase-free water, quantified by spectrophotometry at 260 nm, and examined by agarose gel electrophoresis to check for signs of degradation.

**Saturation study.** We investigated the saturation characteristics of the bovine CBE by varying the glucose concentration gradient across the preparation. The stromal-to-aqueous glucose fluxes (Jw) were studied with glucose concentration gradients of 7.5, 15, 30, 45, and 60 mM across the bovine CBE; their osmolarity was 384, 391, 406, 421 and 436 mosmol/L, respectively. The aqueous glucose concentration was maintained at 7.5 mM while the stromal glucose concentration was changed until the desired gradient was achieved. The difference in osmolarity was balanced with sorbitol. For gradients higher than 15 mM, the glucose concentration was increased in a stepwise manner, at 7.5 or 15.0 mM per increment, until the desired concentration was reached. At this point, the preparation was left to stabilize for another 60 min. Ringer solution containing radiolabeled glucose at the appropriate final concentration was then perfused for another hour before sampling. Glucose fluxes were calculated as described above, and an Eadie-Hofstee relationship was plotted, which allowed the transport kinetics [i.e., kinetic constant (Km) and maximum velocity (Vmax)] to be estimated.

**Statistical analysis.** The statistical analysis was performed using Excel (Microsoft) or GraphPad Prism (version 3.02). All data are expressed as means ± SE; n, sample size. Data were taken around 150 min from the time at which the preparations were mounted. PDt, transepithelial potential difference; Rs, transepithelial resistance; Lsc, short-circuit current.

**Table 2. Electrical parameters of baseline measurement and inhibition studies**

<table>
<thead>
<tr>
<th>Condition</th>
<th>PDt, mV</th>
<th>Rs, Ω·cm²</th>
<th>Lsc, μA·cm²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>−0.52±0.08</td>
<td>95.14±6.17</td>
<td>−5.73±1.07</td>
<td>20</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>−0.42±0.05</td>
<td>91.95±5.77</td>
<td>−4.64±0.48</td>
<td>22</td>
</tr>
<tr>
<td>Cytochalasin B</td>
<td>−0.43±0.18</td>
<td>99.70±11.86</td>
<td>−3.37±1.72</td>
<td>18</td>
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<tr>
<td>Phlorizin</td>
<td>−0.46±0.03</td>
<td>104.40±8.45</td>
<td>−4.44±0.27</td>
<td>16</td>
</tr>
<tr>
<td>Ouabain</td>
<td>−0.14±0.03*</td>
<td>88.99±6.13</td>
<td>−1.63±0.35*</td>
<td>16</td>
</tr>
</tbody>
</table>

*Values are means ± SE; n, sample size. Data were taken around 150 min from the time at which the preparations were mounted. PDt, transepithelial potential difference; Rs, transepithelial resistance; Lsc, short-circuit current. *P < 0.05 compared with baseline.*

**Tissue collection and RNA extraction.** A sector of bovine CBE was isolated as described above. A small portion (0.5 × 0.5 cm) of the pars plana (the posterior aspect of the CBE) was cut from the whole CBE tissue with a sterile scalpel, taking care to avoid contamination from surrounding regions. The tissue was soaked in RNAlater (Ambion, European, Huntingdon, UK), frozen immediately in liquid nitrogen, and stored at −80°C. Tissue samples from the pars plicata (anterior aspect of the CBE) were also isolated. First, the cornea and iris were carefully removed using RNase-free scissors, and then the exposed ciliary processes were harvested using RNase-free forces, whereupon they were soaked in RNAlater, frozen, and stored at −80°C for later use.

**Table 3. Glucose fluxes with and without inhibitors**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Flux Direction</th>
<th>LG, nmol·h⁻¹·cm⁻²</th>
<th>MDG, nmol·h⁻¹·cm⁻²</th>
<th>CMDG, nmol·h⁻¹·cm⁻²</th>
<th>CMDG Flux Inhibition, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Jw (10)</td>
<td>25.8±2.0</td>
<td>189.5±15.1</td>
<td>163.8±14.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jw (10)</td>
<td>263.2±2.3</td>
<td>196.1±14.7</td>
<td>169.8±13.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jw (11)</td>
<td>19.7±1.9</td>
<td>91.2±3.5*</td>
<td>66.5±3.2*</td>
<td>59.4</td>
</tr>
<tr>
<td></td>
<td>Jw (11)</td>
<td>24.6±2.1</td>
<td>93.8±5.3*</td>
<td>69.2±3.7*</td>
<td>59.2</td>
</tr>
<tr>
<td></td>
<td>Jw (9)</td>
<td>27.4±2.45</td>
<td>60.9±9.0*</td>
<td>33.5±9.2*</td>
<td>79.6</td>
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<tr>
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<td>Jw (9)</td>
<td>26.0±4.6</td>
<td>58.2±11.8*</td>
<td>32.1±10.2*</td>
<td>81.1</td>
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<tr>
<td></td>
<td>Jw (9)</td>
<td>22.0±1.9</td>
<td>149.4±9.4*</td>
<td>127.4±7.6*</td>
<td>22.2</td>
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<tr>
<td></td>
<td>Jw (8)</td>
<td>21.3±2.7</td>
<td>155.7±9.6*</td>
<td>134.8±8.3*</td>
<td>20.8</td>
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<td></td>
<td>Jw (8)</td>
<td>28.4±2.5</td>
<td>169.5±18.5</td>
<td>141.1±16.4</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>Jw (7)</td>
<td>27.5±2.0</td>
<td>173.3±10.6</td>
<td>145.8±9.5</td>
<td>14.1</td>
</tr>
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</table>

Flux data are expressed as means ± SE. Jw, stroma-to-aqueous influx; Jsw, aqueous-to-stroma efflux; LG, t-glucose; CMDG, corrected 3-O-methyl-o-glucose. The number of experiment is shown in parentheses. *P < 0.05.
A concentration gradient of D-glucose (CMDG) transport in bovine CBE. A linear function provided a good fit to the data ($r^2 = 0.93$). Error bars represent SE. D/L ratio, CMDG flux-to-LG flux ratio.

Table 4. Glucose fluxes at different glucose concentration gradients

<table>
<thead>
<tr>
<th>Concentration Gradient, mM</th>
<th>LG mmol·h⁻¹·cm⁻²</th>
<th>MDG mmol·h⁻¹·cm⁻²</th>
<th>CMDG mmol·h⁻¹·cm⁻²</th>
<th>D/L Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5 (10)</td>
<td>36.2 ± 8.6</td>
<td>236.6 ± 24.0</td>
<td>200.4 ± 23.6</td>
<td>5.5</td>
</tr>
<tr>
<td>15 (6)</td>
<td>39.6 ± 6.1</td>
<td>306.0 ± 28.0</td>
<td>266.4 ± 27.3</td>
<td>6.7</td>
</tr>
<tr>
<td>30 (6)</td>
<td>44.2 ± 7.6</td>
<td>353.6 ± 31.2</td>
<td>309.4 ± 27.9</td>
<td>7.0</td>
</tr>
<tr>
<td>45 (6)</td>
<td>49.1 ± 3.7</td>
<td>347.1 ± 40.3</td>
<td>298.1 ± 36.4</td>
<td>6.0</td>
</tr>
<tr>
<td>60 (8)</td>
<td>62.4 ± 10.1</td>
<td>383.9 ± 40.6</td>
<td>321.4 ± 34.6</td>
<td>5.2</td>
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</table>

The number of experiments is shown in parentheses. Flux data are expressed as means ± SE. D/L ratio, CMDG flux-to-LG flux ratio.

RESULTS

Electrical parameters. The average $P_D$, short-circuit current ($I_D$), and $R_i$ of the bovine CBE were −0.52 ± 0.08 mV (with the aqueous side consistently negative), −5.73 ± 1.07 μA·cm⁻², and 95.14 ± 6.17 Ω·cm² (means ± SE, $n = 20$), respectively. The electrical parameters were significantly affected by the bilateral addition of 0.1 mM ouabain (Dunnett’s post hoc test, $P < 0.05$), as shown in Fig. 1, but not of phloretin, cytochalasin B, or phlorizin (Dunnett’s post hoc test, $P > 0.05$) (Table 2).

Glucose fluxes across the bovine CBE. The glucose flux measurements in the absence and presence of each inhibitor are summarized in Table 3. No significant net flux (i.e., $J_{sa} - J_{sa}$) was observed for either MDG or LG across the CBE under basal conditions ($P > 0.05$, $n = 10$) or after the addition of different inhibitors ($P > 0.05$, $n = 8–11$). Since MDG moves across the CBE via both the transcellular (transported) and paracellular (including edge damage) routes, LG flux, which mainly diffuses via the paracellular route, can act as a control to reveal the transcellular MDG or corrected MDG (CMDG) flux. The CMDG flux was calculated by subtracting the LG flux from the MDG flux and thus reflects the MDG transport across the bovine CBE. The bovine CBE demonstrated stereospecificity for D-glucose, with CMDG transported 6.3–6.5 times faster than LG under zero glucose concentration gradients. The CMDG flux was markedly decreased with bilateral addition of cytochalasin B (Table 3). Comparatively modest reductions were observed with the addition of phloretin and phlorizin. Ouabain did not cause a significant reduction in the glucose flux (Dunnett’s post hoc test, $P > 0.05$).

Saturation study. In the saturation study, significant depolarization was noted for $P_D$, and $I_D$ with concentration gradients of 30 mM (Dunnett’s post hoc test, $P < 0.001$, $n = 8$), 45 mM (Dunnett’s post hoc test, $P < 0.001$, $n = 8$), and 60 mM (Dunnett’s post hoc test, $P < 0.001$, $n = 8$) compared with the 7.5 mM gradient condition. A summary of these changes is shown in Fig. 2.

Glucose (i.e., MDG and LG) fluxes from the stromal-to-aqueous direction ($J_{sa}$ or influx) were measured and analyzed at five different concentration gradients (Table 4). The stereospecificity of CBE in transporting glucose was maintained across a wide range of concentration gradients, with the highest recorded at 30 mM. Compared with 7.5 mM, glucose gradients of 30 mM or higher caused a significant increase in both MDG and CMDG fluxes (Dunnett’s post hoc test, $P < 0.01$, $n = 6–8$). The LG influx, on the other hand, increased steadily with the concentration gradient (however, this increase did not reach statistical significance, Dunnett’s post hoc test, $P > 0.05$, $n =$...
6–10). The Eadie-Hofstee plot for CMDG showed high linearity, with \( K_m \) and \( V_{\max} \) equal to 5.3 mM and 349.5 nmol·h⁻¹·cm⁻², respectively (Fig. 3).

**mRNA expression of glucose transporter genes.** For the RT-PCR experiments, agarose gels were routinely stained with ethidium bromide. However, where bands were not detected for CBE samples, the more sensitive SYBR gold dye was used (GLUT2 and SGLT experiments). As shown in Fig. 4, GLUT1, GLUT3, GLUT4, and GLUT5 mRNAs were expressed in the CBE tissue (\( n = 3 \) independent experiments) in both the pars plana and pars plicata. However, no GLUT2 gene expression was detected in CBE. In addition, SGLT2 but not SGLT1 gene expression was found in both the pars plicata and pars plana (Fig. 4).

The PCR products for GLUT1–GLUT5 and SGLT1 and SGLT2 were sent for automated sequencing. Their identities were confirmed by BLAST searches against the GenBank database. All BLAST search results demonstrated the best alignment with the respective target mRNA sequence. All the RT-PCR sequences shared 98–100% identity to their target sequences (Table 5).

**DISCUSSION**

We observed an aqueous-side negative PD_{dt} and \( I_{sc} \) across the CBE, similar in direction and magnitude to those reported in previous studies (20, 64). The CBE preparation demonstrated a typical biphasic response to ouabain (35, 67), which is thought to reflect the dual \( Na^+/K^+\)-ATPase (or sodium pump) localization along the basolateral membranes of both the PE and NPE (Fig. 1). This phenomenon has been widely reported and established in other CBE transport models (9, 67). The biphasic response observed in the present study is consistent with other studies (9, 35) and also has been used to test tissue variability involving Ussing chamber experiments of the bovine CBE (64). The unidirectional LG fluxes found in the present study were low compared with a previous study of bovine CBE (64), which indicated that the CBE preparations had good integrity and low leakage.

In the saturation study, the increase in bathing glucose concentration induced a change in membrane potential polarity when the glucose concentration gradient was 30 mM or higher (Fig. 2). The exact mechanism for this change of polarity is unclear. It is anticipated that a high extracellular glucose concentration would shrink the ciliary epithelial cells osmotically. This could trigger volume regulation mechanisms to restore the normal cell size. Such volume regulation changes have been reported in NPE cells (12). A cohort of ion transport events related to cell volume regulation may have contributed to the observed potential changes with high bathing glucose. In any case, the CBE appeared viable at high glucose concentration, since \( Na^+/K^+\)-ATPase transporters remained responsive to ouabain and produced a typical biphasic response as observed in our control CBE preparations.

The present study showed that the glucose transport of bovine CBE was saturable, as estimated by the Eadie-Hofstee plot (Fig. 3), and stereospecific (Tables 2 and 3). It also was sensitive to phloretin, cytochalasin B, and phlorizin inhibition (Table 3). As expected, cytochalasin B, which is the more potent inhibitor, showed greater inhibition of facilitative glucose transport than phloretin, as is the cases for bovine RPE (63) and frog lens (40). Since phloretin and cytochalasin B are potent inhibitors of GLUT-type transporters (38–40), we may conclude that passive facilitated transport is the major mode of glucose transport across the bovine CBE. Since the glucose concentration of the aqueous humor is lower than that of the blood stream (19), it is reasonable to expect that passive facilitated diffusion is sufficient to supply glucose into the anterior eye. In addition, there was no significant net transport found in either direction of glucose movement, which further indicates the importance of passive transport rather than active transport of glucose.

Nevertheless, a significant reduction (20%) in glucose flux was produced by phlorizin, an inhibitor of active glucose transport. Furthermore, the CBE showed expression of mRNA for SGLT2, a member of the sodium-dependent glucose transporter family (41, 48). The presence of SGLT2 mRNA in the
bovine CBE was surprising, given the lack of net glucose transport we observed. It is possible that the net glucose flux across the CBE was so small that the current electrophysiological system failed to identify it. However, we also noted that no changes in the electrical parameters of the CBE were observed after the addition of phlorizin, which usually produces electrical changes in active glucose transporting systems (8, 29). Another possible explanation for the inhibitory effect observed with phlorizin relates to the fact that it can be converted to phloretin (usually via the action of endogenous lactase phlorizin hydrolase) and thereby exert an inhibitory effect on glucose transport via inhibition of GLUT transporters (6, 42, 65). Alternatively, it is possible that SGLT2 mRNA is not translated in CBE cells or that it is expressed but does not function in actively transporting glucose. For example, in bovine cortical arteries, where GLUT1 has been found to be responsible for basal glucose transport, the high-affinity transporter SGLT1 acts as a molecular sensor for coping with stresses such as hypoglycemia (49). In addition, SGLT3 has been found to act as a “nutrient-sensing device,” which mediates glucose-induced depolarization of the membrane potential but does not possess transport activity, when expressed in Xenopus laevis oocytes (15). SGLT3 also has been suggested to act as a glucose sensor in cholinergic neurons in regulating muscle activity (15). Finally, sugar entry via GLUT1 and SGLT3 has been proposed to act as a glucose sensor in the GLUTag cell (25). Therefore, it is possible that SGLT2 is expressed in the bovine CBE but that its role relates to that of a glucose sensor or in detecting or responding to cellular stress.

RT-PCR suggested that several GLUT-type transporter transcripts were present in the bovine CBE. The pars plicata and pars plana showed no particular difference in their gene expression profile, but the pars plana appeared to have a relatively higher mRNA expression level overall. The presence of GLUT1 and GLUT3 mRNA is not surprising, since GLUT1 is regarded as one of the biochemical signatures of “barrier” tissues such as the various ocular epithelia (24, 28). Together with GLUT3, GLUT1 takes part in the basal cellular glucose uptake in a number of mammalian tissues (43, 45). The presence of GLUT4 might suggest the presence of insulin-responsive glucose uptake in bovine CBE, as observed in cerebellum (33, 52). The existence of GLUT5 could indicate the presence of fructose transport along with glucose transport, since GLUT5 has a significantly higher transport capacity for fructose than glucose (47, 54).

In the RT-PCR reaction for SGLT2, a consistent pattern of double bands was seen, with the expected band showing the higher intensity. Such a pattern was conserved across a wide range of PCR annealing temperatures (63–68°C; data not shown), which suggests the chance of nonspecific binding was low. One possibility is that the double bands arose due to alternative splicing of SGLT2; however, this was not investigated directly. Alternative splicing of GLUT9, GLUT11, and GLUT14 has been reported in kidney and placenta (2), skeletal muscle and heart (70), and testis (69), respectively. However, similar splicing of other glucose transporter isoforms has not been reported. Alternate splicing has been reported in a range of different cell types (37, 50), but it is not directly related to particular abnormalities or disease.

In conclusion, glucose transport across the bovine CBE is mainly mediated by a bidirectional carrier-facilitated transport mechanism. This is similar to that of the blood-retinal barrier, which shares the same embryological origin as the BAB during development. The finding of SGLT2 mRNA expression in the CBE was surprising and therefore means that a potential role for active glucose transport in the CBE cannot be ruled out. However, if present, active glucose transport is unlikely to be important under basal conditions. Together with the electrophysiological results, these data suggest that if SGLT2 is indeed functionally active, it may be involved in an aspect of glucose homeostasis, such as a glucose concentration sensor, rather than active transport.

**ACKNOWLEDGMENTS**

We thank K. K. Li for technical assistance and advice on the experiment.

**GRANTS**

This work was supported by Hong Kong Polytechnic Research Grant 8-ZF84, GW079.

**REFERENCES**


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