Case 17
A Possible Mechanism for Blindness Associated with Diabetes: Na⁺-Dependent Glucose Uptake by Retinal Cells

Focus concept
Glucose transport into cells can influence collagen synthesis, which causes the basement membrane thickening associated with diabetic retinopathy.

Prerequisites
X Transport proteins.
X Na⁺K⁺ATPase and active transport.

Background
In the retina of the eye, there are equal amounts of endothelial cells and cells called pericytes. The two types of cells are associated with one another and may communicate with each other. The investigators were particularly interested in pericytes because it had been observed that pericyte damage and basement membrane thickening occur during the early stages of diabetic retinopathy. Diabetic retinopathy eventually leads to blindness.

The investigators were interested in the factors responsible for causing pericyte damage. They noted that cells in the kidney that were similar in function to the pericytes possessed a sodium-coupled glucose transporter (SGLT), and they hypothesized that the pericytes might also have such a transporter. They hypothesized that such a transporter, if it existed, would facilitate and perhaps regulate the entry of glucose into the cell. Next, the investigators determined if entry of glucose into the cell was correlated with collagen synthesis. They also investigated several inhibitors of the glucose transport process.

Questions
1. The investigators cultured pericytes and endothelial cells separately and measured their ability to take up glucose. Glucose uptake was measured both in the presence of sodium ions (provided by sodium chloride) and in the absence of sodium (choline chloride was added in order to maintain constant osmolarity). The results are shown in Figure 17.1. What is your interpretation of these results?
2. Next, the investigators measured the effect of increasing sodium concentration on glucose uptake in both pericytes and endothelial cells. The results are shown in Figure 17.2. What is your interpretation of these results? What information is conveyed by the shape of the curves?

**Figure 17.1**: Effect of Na⁺ on glucose uptake by two different types of retinal cells. The concentrations of both NaCl and choline chloride were 145 mM (based on Wakisaka, et al., 1997).

**Figure 17.2**: A dose-dependent response for the sodium-dependent uptake of glucose by pericytes and endothelial cells (based on M. Wakisaka, et al., 1997).
3. Transport kinetics through protein transporters can be described using the language of Michaelis-Menten. The transported substance binding to its protein transporter is analogous to the substrate binding to an enzyme. Thus, $K_M$ and $v_{max}$ values can be determined for protein transporters. Use the information provided in Figure 17.3 to estimate the $K_M$ and $v_{max}$ for glucose uptake by pericytes in the presence and absence of sodium. What information is conveyed by these values? Have the investigators demonstrated convincingly that a SGLT exists in pericytes? What about endothelial cells? Explain, using the data to support your answers.

Figure 17.3: Lineweaver-Burk plots for the uptake of glucose by pericytes in the presence (145 mM NaCl) or absence (145 mM choline chloride) of sodium ions (based on Wakisaka, et al., 1997).
4. The activity of the SGLT in pericytes was investigated in the presence of inhibitors. Radioactively labeled glucose was added to cultured pericytes, in the absence (control) or presence of several potential inhibitors. The transport experiments were carried out both in the presence and absence of sodium ions. The results are shown in Figure 17.4.

a. What is the effect of sugars such as galactose and 2-deoxyglucose on glucose transport?

b. What is the effect of 0.2 mM phlorizin on glucose transport?

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**Figure 17.4**: Effect of inhibitors on glucose uptake by pericytes in the presence and absence of sodium ions. The sugars were added at a concentration of 5 mM, while the phlorizin was added at a concentration of 0.2 mM (based on Wakisaka, et al., 1997).
5. Next, the investigators wanted to explore the relationship between glucose uptake and collagen synthesis. They added increasing amounts of glucose to cultured pericytes and then measured the relative density of the cells as a result. (They confirmed in a separate immunoblotting experiment that increased collagen synthesis was responsible for the increased cellular density.) The results are shown in Figure 17.5. How do you interpret these data?

![Figure 17.5](image-url)

**Figure 17.5**: Effect of increasing glucose concentration of the synthesis of Type IV and Type VI collagens (based on Wakisaka, *et al.*, 1997)
6. The effect of the drug phlorizin on glucose consumption and relative cellular density (compared with 5 mM glucose) was examined. The results are shown in Figure 17.6.
   a. What effect does phlorizin have on glucose consumption at the two different concentrations of glucose?
   b. What effect does phlorizin have on relative density of the cells at the two glucose concentrations?

![Image of Figure 17.6](image.png)

**Figure 17.6**: Effect of phlorizin on glucose consumption (left) and collagen synthesis (right). Based on Wakisaka, *et al.*, 1997.

7. Consider the diabetic vs. the nondiabetic patient. Why do you think that retinopathy is more likely to occur in the diabetic patient? Describe the sequence of biochemical events leading to pericyte cell damage. What steps might be taken to decrease the incidence of diabetic retinopathy? Explain your answer, using the experimental results presented here.

Reference