Onset of Changes in Glucose Transport Across Ocular Barriers in Streptozotocin-Induced Diabetes

Joseph DiMattio, Jose A. Zadunaisky, and Norman Altszuler

In a previous study, measurements were made of facilitated and passive transport of glucose, using \(^{3}\)H-3-O-methyl-D-glucose and \(^{14}\)C-L-glucose, respectively, across blood-aqueous and blood-vitreous barriers in long-term streptozotocin-diabetic rats. It was found that passive transport was increased, while facilitated transport was decreased, possibly due to saturation of the transport system. The present study examines the appearance of these changes in glucose transport at various times following streptozotocin (STZ) injection. Passive transport, as indicated by the L-glucose rate constant, began to increase at about 10 days following induction of diabetes, stabilized at the elevated rates at 50–60 days and persisted during the 170-day period of observation. Rate constants for \(^{3}\)H-3-O-methyl-D-glucose transport decreased within 1 day following induction of diabetes. Prevention of hyperglycemia by insulin treatment upon onset of diabetes prevented the latter changes ruling out a direct effect of STZ. Glucose infusion into normal rats produced a similar decrease in 3-O-methylglucose transport constants suggesting that hyperglycemia was responsible for the early decrease in facilitated transport found in the diabetic rats. It is speculated that increased passive transport of glucose may reflect an early loss in ocular barrier integrity. The later decrease in carrier facilitated transport cannot be explained by hyperglycemia alone and, thus, a loss in carrier function is suggested. Despite a decrease in facilitated transport, absolute glucose entry rates are increased in the diabetic due to elevated plasma glucose, which serves as an inward driving force, due to the significantly increased entry of glucose by the passive route.

Materials and Methods

All experiments were carried out in male albino Sprague-Dawley rats 200–300 g, under sodium pentobarbital anesthesia (50 mg/kg, ip) with methods conforming to the ARVO Resolution on the Use of Animals in Research. Streptozotocin (65 mg/kg) was dissolved in citrate buffer solution (0.09 M in Saline, pH 9.5) immediately before injection into a tail vein. Plasma glucose was elevated within 24 hr and remained elevated, as determined by periodic monitoring of urine or blood using respectively, Keto-Diastix (Miles Laboratories; Elkhart, IN) or glucose oxidase, using the Beckman Glucose Analyzer (Beckman Instruments; Fullerton, CA).

The animals received no insulin except in some acute experiments as indicated, and in such cases all animals had demonstrated urinary glucose prior to insulin administration. All other animals had plasma-glucose levels higher than 300 mg/dl at the time ocular transport was studied.

Each experiment was performed using double-labeling with \(^{3}\)H-3-O-methyl-D-glucose and \(^{14}\)C-L-glucose, thus allowing the determination of transport rates of both D- and L-glucose in each test animal. The procedure for determining blood ocular transport rate has been outlined in detail previously. In sum-
Table 1. Acute effects of streptozocin-induced diabetes on ocular glucose transport in rats

<table>
<thead>
<tr>
<th>Days post-STZ</th>
<th>Plasma glucose (mg/dl)</th>
<th>Blood-aqueous</th>
<th>3-O-methylglucose</th>
<th>L-glucose</th>
<th>Blood-vitreous</th>
<th>3-O-methylglucose</th>
<th>L-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (14)*</td>
<td>154 ± 18†</td>
<td>0.067 ± 0.006</td>
<td>0.013 ± 0.002</td>
<td>0.042 ± 0.004</td>
<td>0.007 ± 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>340</td>
<td>0.051</td>
<td>0.014</td>
<td>0.033</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>360</td>
<td>0.053</td>
<td>0.013</td>
<td>0.030</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>426</td>
<td>0.048</td>
<td>0.013</td>
<td>0.026</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>480</td>
<td>0.053</td>
<td>0.015</td>
<td>0.031</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>460</td>
<td>0.052</td>
<td>0.014</td>
<td>0.032</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>484</td>
<td>0.044</td>
<td>0.013</td>
<td>0.033</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>510</td>
<td>0.059</td>
<td>0.017</td>
<td>0.037</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean + SD</td>
<td>444 ± 76†</td>
<td>0.050 ± 0.007</td>
<td>0.013 ± 0.002</td>
<td>0.033 ± 0.004</td>
<td>0.008 ± 0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of experiments.
† Mean + SD.
‡ P > 0.05; statistical analysis based on Student’s t-test.

mary, a bolus injection of labeled material is introduced into a cannulated femoral vein of an anesthetized Sprague Dawley rat. From 0–18 min, small samples of blood (100 μl) are withdrawn at about 2-min intervals and replaced with saline. At 18 min, a final sample of blood is obtained, the eyes are quickly removed and measured volumes (10–25 μl) of aqueous and vitreous humor are obtained. The 14C and 3H in each sample of plasma, aqueous humor, and vitreous humor is counted by liquid scintillation spectrometry with appropriate corrections for channel spillover and quenching performed with computer assistance to yield concentrations of label in DPM/ml. The plasma concentration (Cp) data are plotted with time and an equation of the form:

\[ C_p = A + B e^{-bt} + C e^{-bt} \]  

(Eq. 1)

is graphically fit to the data. The constants A, B, C, b1 and b2 are determined from a best fit to the data. Ocular concentrations obtained at 18 min, along with the determined plasma constants that define the blood function and previously determined steady state values were analyzed to give the transport constants, Ki, using a model described previously.1,3 The model accounts for passive, active and bulk transport of fluid into the ocular compartments, but it can be simplified for L-glucose and 3-O-methyl-D-glucose transport to give the following equation:

\[ \frac{dC_A}{dt} = K_i C_p - K_o C_A \]  

(Eq. 2)

where \( C_A \) and \( C_p \) refer to concentrations of test substance in aqueous (or vitreous) and plasma, respectively. For both L-glucose and 3-O-methyl-D-glucose steady state values of aqueous to plasma and vitreous to plasma concentration ratios approach 1.0, thus \( K_i = K_o \) and consequently only \( K_i \) is used here. Evidence for this has been presented previously.1 Rate constants for entry and exit, \( K_i \) and \( K_o \) (min⁻¹), respectively, are determined by a computer solution to the above equation and reflect a fractional change in ocular compartment concentration occurring per unit of time. Thus, the rate constants measure how fast the ocular compartment concentration of labeled substance is changing and since neither tracer is utilized in the body the rate constants assess barrier function and integrity. Absolute mass transport entry rates are directly proportional to both the transport rate constant, \( K_i \), and the prevailing plasma glucose concentration, \( C_p \).

Results

As shown in Table 1, plasma glucose levels rose significantly as early as 1 day after streptozotocin injection. This is similar to observations reported by others in rats.5-7 One day after streptozotocin, there also is a significant decrease in 3-O-methylglucose transport constants for both ocular barriers. This early decrease probably reflects decreased availability of transport carrier rather than a direct impairment of transport inasmuch as a similar decrease in transport is produced in normal rats made hyperglycemic by
Table 2. Ocular glucose transport following insulin treatment within 7 days of streptozotocin-induced diabetes

<table>
<thead>
<tr>
<th>Plasma glucose mg/dl</th>
<th>3-O-methylglucose</th>
<th>L-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control normal (3¶)</td>
<td>132 + 21§</td>
<td>0.071 ± 0.005</td>
</tr>
<tr>
<td>STZ-Diabetic + insulin (6)</td>
<td></td>
<td>111 ± 12</td>
</tr>
</tbody>
</table>

* Rate of transport across blood-aqueous barrier.  
† Rate of transport across blood-vitreous barrier.  
‡ Number of animals.  
§ Mean + SDM.  
|| Animals received last insulin injection about 3 hr prior to experiment.

Table 3. Ocular glucose transport at various periods of streptozotocin-induced diabetes in rats

<table>
<thead>
<tr>
<th>Days</th>
<th>Blood-aqueous transport Kt (min⁻¹)</th>
<th>Blood-vitreous transport Kv (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(16)*</td>
<td>(7)</td>
</tr>
<tr>
<td>0</td>
<td>34 + 20†</td>
<td>N.S.†</td>
</tr>
<tr>
<td>1-10</td>
<td>420 + 61</td>
<td>N.S.</td>
</tr>
<tr>
<td>11-20</td>
<td>454 + 45</td>
<td>N.S.</td>
</tr>
<tr>
<td>21-30</td>
<td>464 + 30</td>
<td>N.S.</td>
</tr>
<tr>
<td>31-40</td>
<td>451 + 40</td>
<td>N.S.</td>
</tr>
<tr>
<td>51-60</td>
<td>490 + 37</td>
<td>N.S.</td>
</tr>
<tr>
<td>70-80</td>
<td>475 + 55</td>
<td>N.S.</td>
</tr>
<tr>
<td>152-172</td>
<td>510 + 50</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* Number of animals; data for days 159-172 taken from previous study (1).  
† Mean + SDM.  
‡ P > 0.05; all statistical comparisons are made with control normal animals (days 0).
Fig. 1. Rates of facilitated and passive transport of glucose across the aqueous humor in rats in normal (N) state and at various times following injection of streptozotocin (at 0 time). The actual mean values ± SD and statistical evaluations are shown in Table 3. The contribution of passive transport was subtracted from the observed facilitated transport and such corrected facilitated transport is shown by the dotted points in each bar and taken as a measure of facilitation of transport.

The mean value for the 10-day period, which is not significantly different from normal. However, passive transport is seen to increase thereafter, as shown in Table 3. It is increased significantly in the 11–20-day period and continues to rise slowly until it attains relatively stable values at 50–60 days and remains elevated for the remainder of the experimental period. These changes are due to the diabetic state because control normal rats kept under similar experimental conditions and similar time period did not show any changes in L-glucose transport across either the blood–aqueous or vitreous barriers (data not shown).

Table 3 also shows the values for 3-O-methylglucose transport during the course of the 172-day period of observation. As already discussed, the initial decrease in facilitated transport is attributed to saturation of glucose transport due to the hyperglycemia. There is a brief upswing in the transport rate at 21–40 days. This change occurs concomitantly with the rise in passive transport, which probably contributes to the observed rises. However, beginning at 40–50 days, the facilitated transport decreases again and remains low for the duration of the study, despite the persistent elevation in passive transport.

Table 3 also gives the ratio of D/L glucose transport rates. It is evident that in the normal rat, glucose transport occurs largely by facilitated diffusion. The D/L ratio decreases in the diabetic due to a decrease in D-glucose and increase in L-glucose transport.

Figures 1 and 2 depict graphically the glucose transport rates at the two ocular barriers. The pattern of changes in transport of 3-O-methylglucose and L-glucose are clearly evident. Since passive transport is available to both sugars, subtraction of passive transport from the observed transport of 3-O-methylglucose may give a more appropriate measure of facilitated glucose transport. Such corrected transport rates are shown by the line drawn across the bar graphs. It can be seen that the rise in measured facilitated transport, observed or corrected, occurring at 21–40 days is not sustained, and, indeed, the rates decline despite the continued increased rates of passive transport. The corrected transport rates in the final period are significantly lower ($P < 0.001$) than those at 30–40 days suggesting a progressive impairment of carrier function.

Discussion

A main finding in this study is that passive glucose transport begins increasing soon after the onset of diabetes. The rise begins at about 10 days following induction of diabetes, continues slowly up to 50–60 days, and stabilizes at the elevated values beyond that period. The increase in permeability to glucose occurs at both the aqueous and vitreous barriers. This is in agreement with the observations that there is greater passive penetration of fluorescein into the vitreous humor in diabetic humans than in normal subjects.8,9 It is of in-
Interest that although hyperglycemia occurs within hours after STZ injection, significant increases in passive transport of glucose begin to occur only after 10 days. The reasons for the slow changes in permeability to glucose are not known. Conceivably, this may involve development of structural changes or biochemical adaptations, but these possibilities need to be explored.

These altered rates of permeability to L-glucose may reflect changes either in the vascular supply to the eye or in the epithelial barriers of the eye compartments. It is unlikely that the increases in passive transport of glucose into the eye compartments involve only changes in vascular permeability. This is reasoned from the fact that the formation and composition of the aqueous and vitreous fluids appears to be regulated by tight barriers of epithelium. At the blood–aqueous barrier, the double layered ciliary epithelium and tight junctions of the unpigmented layer block passive movement of molecules, including glucose. Although the epithelial tissues bordering on the aqueous do not completely envelop the aqueous, they do provide a major protective barrier. On the other hand, the vascular input to aqueous secretion is derived from capillaries at the ciliary process, but these capillaries are well-fenestrated allowing free, passive movement and, therefore, it is unlikely that they would contribute to further increase aqueous barrier permeability. In the case of the iris, however, the barrier between the blood vessels in the stroma and the aqueous is achieved by a tight endothelium lining of the capillaries as well as by the restraint provided by the posterior epithelium of the iris.10 It is conceivable that changes in permeability to glucose could occur in these capillaries and, thereby, contribute to the observed increase in permeability to glucose between blood and the aqueous, but, to date, there is no evidence for this possibility.

Similarly, the retinal pigment epithelium regulates the environment of the outer retina and forms a barrier of tight junctions between the fenestrated vessels of the choroid and the retina. Thus, changes in permeability of this epithelial barrier could contribute to the increased permeability of the vitreous barrier. The inner retina also is supplied with vessels with tight junctions and a change in their permeability could possibly be a contributory factor.

The mechanism whereby persistent hyperglycemia may alter cellular function remains unclear. If tissue concentration of glucose exceeds that of the plasma, it could exert an osmotic effect as has been suggested by Kinoshita et al.11 However, in the diabetic, the retina–plasma ratio of glucose concentration is probably unchanged from the normal, which may argue against an osmotic effect. Another explanation for tissue damage involves increased polyol pathway activity seen in diabetes.12 The hyperglycemia would be expected to provide more glucose precursor for the polyol pathway, resulting in increased tissue accumulation of less permeable glucitol (sorbitol) and a parallel increase in water content as observed in the lens. However, it is not clear at present that the “osmotic theory” fully explains sugar cataractogenesis since a number of other plausible hypotheses have been put forth including the suggestion that decreased availability of NADPH could lead to a limited ability of the lens to dispose of damaging peroxides.13 Moreover, the decrease in glucose metabolism observed in lens exposed to high carbo-
hydrate concentrations could contribute to alterations in sodium-dependent water movement across the lens epithelium.\textsuperscript{13} Despite uncertainty of the mechanism, our work suggests that early permeability changes tend to enhance glucose entry into the ocular compartments and expose intraocular tissues to damaging high glucose concentrations.

Another possible mechanism for the altered permeability may involve nonenzymatic glycosylation, which has been shown to occur in vivo in a variety of proteins including hemoglobin, red cell membrane protein, collagen, lens crystallin, serum proteins and nerve myelin.\textsuperscript{14}

Facilitated transport of glucose also appears to show changes in the diabetic animals, although these are more difficult to interpret. There are several factors that influence the measurement of transport of \(^{(3}H\) 3-O-methylglucose. Since the tracer uses the same transport system as endogenous D-glucose, it will be affected by the prevailing plasma glucose concentration. Indeed, as we have reported previously,\textsuperscript{1} increasing plasma glucose levels in normal rats by glucose infusion decreased the rate of transport of 3-O-methylglucose to about 60\% of control level. The observed decrease in tracer transport in the diabetic rats reported here may have a similar explanation.

Another complication in the measurement of facilitated transport is the extent of contribution of the passive movement of glucose. We presume that an increase in passive movement or permeability to glucose also would increase transport of 3-O-methylglucose. Since we made independent measurements of glucose permeability, using \((^{14}C\)-L-glucose, we can subtract these rates from those obtained using 3-O-methylglucose and obtain a more reliable measure of facilitated transport. Such values are represented in Figures 1 and 2 by the solid lines.

Facilitated transport of glucose, total or corrected, is shown to decrease immediately upon induction of diabetes, and this result is attributed to the hyperglycemia and saturation of the transport system. In the 10–40-day period, facilitated transport is seen to rise somewhat. This may be due, in part, to the concomitant rise in passive transport. With time, 3-O-methylglucose decline to initial diabetic values and upon subtracting the passive transport, the corrected facilitated transport is lower than at the onset of diabetes. This finding is viewed as suggestive of a decline in carrier facilitated transport in long-term diabetes. Nevertheless, since plasma glucose concentration, which is the driving force for glucose entry, is more than three times normal as early as 1 day post-streptozotocin, the mass rate of glucose entry is still significantly elevated and a small loss in carrier function will have little consequence in determining intraocular glucose concentration.

Although the preceding discussion has emphasized the possible role of hyperglycemia and increased permeability to glucose as contributing to the complications of long-term diabetes, other factors may also play a role, such as changes in the vasculature,\textsuperscript{15–17} with particular emphasis on thickening of the basement membrane.\textsuperscript{18–20} Such thickening in small blood vessels\textsuperscript{21,22} and kidneys\textsuperscript{23,24} is well documented, and this finding has been associated with increased permeability. Basement membrane thickening in diabetics is generally a slow process and is a major factor in the microangiopathy of kidney, but it is uncertain if it has much influence on the function of other organs.\textsuperscript{20} Presumably, the increase in permeability seen in the present study can lead eventually to basement membrane abnormalities and altered membrane transport functions. It is of interest that in the tissues most affected in chronic diabetes, eg, nervous tissue, kidney, blood vessels, transport of glucose is not dependent on insulin\textsuperscript{25} and, thus, may be influenced largely by the prevailing plasma glucose.\textsuperscript{21,26}

Regulation of glucose transport in the eye and a possible role of insulin in this process is still unsettled.\textsuperscript{27} The early studies by Ross\textsuperscript{28,29} and Harris\textsuperscript{30} proposed an effect of insulin on glucose transport in the lens, but others\textsuperscript{31,32} have questioned these findings. The present study shows that the carrier facilitated glucose transport is decreased in the long-term diabetic animal. Since the magnitude of this defect is greater at the later stage of the diabetes than in the early stage, it would appear that it is not directly related to the lack of insulin. Thus, the role played by insulin in glucose transport in the eye, if any, requires better substantiation than has been available thus far.

**Key words:** glucose transport in eye, diabetic rats, facilitated glucose transport, passive glucose transport

**Acknowledgment**

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**References**