Glucose Transport Across Ocular Barriers of the Streptozotocin-Diabetic Rat

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SUMMARY

The transport kinetics across the plasma-aqueous and plasma-vitreous barriers were studied in normal and long-term streptozotocin-diabetic rats, using trace amounts of [^{14}C]-L-glucose and [^{3}H]-3-O-methyl-D-glucose. The former is passively transported while the latter uses the same transport-facilitating system as D-glucose. Transport rates of L-glucose were significantly higher in the diabetic rats, with ocular entry rates from the plasma being increased by 69% across both barriers. Thus, the data indicate that in experimental diabetes the passive permeability of the blood-ocular barriers is significantly increased.

By contrast, calculated transport rate constants for 3-O-methyl-D-glucose, when adjusted for the hyperglycemia and the increased passive glucose movement, are not altered in the diabetic animal. Nevertheless, there is actually more mass D-glucose movement due to the prevailing hyperglycemia. The present study suggests that although streptozotocin diabetes alters plasma-ocular glucose transport, there is no direct impairment of glucose carrier function.

Alterations in transport occurred at both ocular barriers, suggesting that involvement is general and that both the retinal pigment epithelium and the ciliary epithelium may be affected by the diabetes. It is unknown whether the increase in passive movement is related to the prevailing hyperglycemia or to insulin deficiency or other unknown factors. DIABETES *30*:903–906, November 1981.

Previous studies have demonstrated that in species as divergent as the dogfish shark and Sprague-Dawley rat, D-glucose crosses both the blood-aqueous and blood-vitreous barriers of the eye quite readily by a mechanism consistent with facilitated diffusion.[1,2] These studies suggested that passive and facilitated transport (nonpassive) could be characterized by the transport of stereoisomers L- and D-glucose, respectively. Thus, the D-glucose transport rate is considered indicative of functional transport activity, whereas L-glucose transport parallels passive movement, being solely dependent on molecular size, lipid solubility, and passive membrane characteristics without a specific transport mechanism.

In addition, we have previously reported that 3-O-methyl-D-glucose is also transported via facilitated diffusion and likely by the D-glucose transport system.[2] It has an advantage over D-glucose in that it is essentially not metabolized after being transported into the ocular fluids. In the present study L-glucose and 3-O-methyl-D-glucose transport rates were used to characterize the blood-ocular barriers of normal and streptozotocin-induced diabetic rats.[1]

It was found that in rats with diabetes of long standing (about 160 days), the passive transport of L-glucose occurred at significantly greater rates than in control animals. In addition, although the apparent rates of transport from the plasma compartment to the intraocular fluid compartments of 3-O-methyl-D-glucose was decreased, this decrease could not be attributed to any impairment of carrier function but was due likely to carrier saturation.[2] The apparent overall plasma-ocular transport rate of D-glucose consisting of passive and carrier-mediated components is complex, with the diabetes affecting primarily passive permeability and with little if any alteration in carrier transport. The similarity in transport changes at vitreous and aqueous barriers suggests that both the ciliary epithelium and the retinal pigmented epithelium may be affected by the induced diabetic state.

MATERIALS AND METHODS

Normal male albino Sprague-Dawley rats weighing 200–300 g were used as controls and for induction of diabetes with streptozotocin. Each of 20 rats, fasted for 24 h, was anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then injected via a tail vein with streptozotocin (65 mg/kg), dissolved immediately before injection in citrate buffer solu-

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tion (0.09 M in saline, pH 4.5). Plasma glucose was elevated at 2 days following injection and remained elevated as judged by intermittent monitoring of urine or blood glucose using Keto-Diastix (Miles Laboratories, Elkhart, Indiana) and directly by glucose oxidase,[3] using the Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California). The animals received no insulin and only animals with plasma glucose values above 350 mg/dl were used.

EXPERIMENTAL PROCEDURE

The femoral vein of anesthetized rats was cannulated using polyethylene tubing (P.E. 50), which was connected to a two-way stopcock to simplify blood removal or injection of materials. Heparin (25 IU/kg) was administered to minimize blood clotting. At a designated time zero, a calibrated bolus of labeled material was injected in 0.5 ml of saline. Small samples of blood (~100 μl) were removed at 2, 4, 7, 10, 15, and 18 min and the volume replaced at each time with saline. The animal was then killed with a dose of pentobarbital and both eyes quickly enucleated. A sample of aqueous humor (5–15 μl per eye) was obtained through the cornea and an aliquot prepared for isotope counting. The vitreous humor was then removed by a cut through the back of the eye and a pooled 20–30-μl sample from both eyes was collected for isotope counting. Each experiment provided a plasma concentration curve and measurements of aqueous and vitreous concentrations at the end of the test period.

The plasma-ocular transport rates, using [^{3}H]-3-O-methyl-D-glucose and [^{4}C]-L-glucose, were measured as described in detail previously.[2] Rate of transport parameters, K_i (min^{-1}) and K_o (min^{-1}), measuring the apparent entry and exit rates of labeled test molecules into the aqueous and vitreous fluids of the eye from the plasma compartment, were determined using the plasma-ocular humors model presented here in brief.[4]

The model assumes that transport to and from the ocular compartment can occur by passive diffusion, active secretion, or bulk absorption. The resulting system equation is:

$$\frac{dC_A}{dt} = K_DC_P - K_DC_A + K_FnC_P - K_FC_A \quad (1)$$

where C_A and C_P are tracer concentration variables for plasma (P) and ocular humor (A), and K_D (min^{-1}) is the diffusion rate constant. K_F (min^{-1}), the secretion constant, is assumed to be equal to the bulk absorption constant since the eye compartment volume remains relatively constant. The concentration of newly secreted fluid is taken as nC_P, which is a simple linear function of plasma concentration. At steady state,

$$\left(\frac{dC_A}{dt}\right)_{t\to\infty} = 0 \text{ and } \frac{C_A}{C_P} = \frac{K_Fn + K_D}{K_F + K_D} = \frac{K_i}{K_o} \quad (2)$$

The steady-state value of C_A/C_P used for both L-glucose and 3-O-methyl-D-glucose transport rate calculations was 1.0, which implies that neither substance is actively secreted against a concentration gradient; experimental support for this has been presented elsewhere.[3]

Thus, n, defined as the concentration of newly secreted ocular fluid divided by the plasma concentration, is for both L-glucose and 3-O-methyl-D-glucose equal to 1.0 and $K_i = K_o$. The model equation in this case simplifies to

$$\frac{dC_A}{dt} = K_iC_P - K_oC_A \quad (3)$$

After a bolus injection of labeled test material was introduced into the plasma, the concentration of label was determined periodically and these data were fit graphically to an equation of the form:

$$C_P = A + Be^{-b_1t} + Ce^{-b_2t} \quad (4)$$

From an estimation of C_A/C_P at steady state, the plasma concentration constants A, B, C, b_1, and b_2, and determined ocular concentrations at a specific time, Eq. (4) can be solved to yield rate constants K_i and K_o.

Each experiment was performed using double labeling [^{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 and acting as an internal control.

RESULTS

Table 1 shows results obtained with [^{14}C]-L-glucose and [^{3}H]-3-O-methyl-D-glucose introduced simultaneously into individual test animals. Measurements on diabetic rats were performed 160 ± 8 days after the administration of strepto-

TABLE 1
The rates of transport of L-glucose and 3-O-methyl-D-glucose across the ocular barriers of control and streptozotocin-diabetic rats

	Plasma glucose (mg/dl)	Aqueous		Vitreous	
		C_A/C_P*	K_i (min^{-1})	C_V/C_P*	K_i (min^{-1})
[^{14}C]-L-glucose					
Control (14)	158 ± 4† (144–168)	0.372 ± 0.020	0.013 ± 0.002	0.209 ± 0.011	0.0068 ± 0.0016
Diabetic (11)	471 ± 31 (396–532)	0.596 ± 0.038	0.022 ± 0.003	0.298 ± 0.028	0.0115 ± 0.0014
[^{3}H]-3-O-methyl-D-glucose					
Control (14)	158 ± 4 (144–168)	0.918 ± 0.023	0.067 ± 0.006	0.697 ± 0.021	0.042 ± 0.004
Diabetic (11)	471 ± 31 (396–532)	0.723 ± 0.051	0.047 ± 0.005	0.584 ± 0.040	0.028 ± 0.004

* Experimentally determined concentration ratios at 18 min. Concentration in aqueous (C_A), vitreous (C_V), and plasma (C_P).
† The number of experiments in parenthesis. Values are reported as means ± SDM.

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zotocin. It can be seen that in the diabetic animals, 3-O-methyl-D-glucose transport constants based on measurements with radiolabeled compound are decreased by 30% across the plasma-aqueous and 33% across the plasma-vitreous barriers. When elevated glucose levels are taken into account, unidirectional rates of unlabeled glucose, which depend on the product of K_i and C_p, are increased by 108% across the aqueous and 99% across the vitreous barriers, assuming that the diffusion characteristics (transport area) of the diabetic animal have not changed. By contrast, ocular entry constants for L-glucose were significantly ($P < 0.001$) greater in the diabetic animal than in control animals, being increased by 69.1% and 69.2% across the aqueous and vitreous barriers, respectively. Table 1 also shows that plasma glucose levels of the diabetic animals obtained at the time of ocular transport measurements were about three times greater than those of control animals.

The ratio of the transport rates of D- and L-glucose as seen in Figure 1 is a concise measure of barrier selectivity. If movement of glucose from blood to the ocular fluids were only passive, this ratio would be 1.0. The D/L ratio of 5 or greater in the normal animal attests to the importance of facilitative transport in the normal state. The diabetic animal exhibits a decreased ability to selectively transport D-glucose from plasma to the ocular fluids. The D/L transport ratio across the aqueous barrier in diabetic animals is only 40.4% that of normal animals and, similarly, it is 38.7% of control across the vitreous barrier.

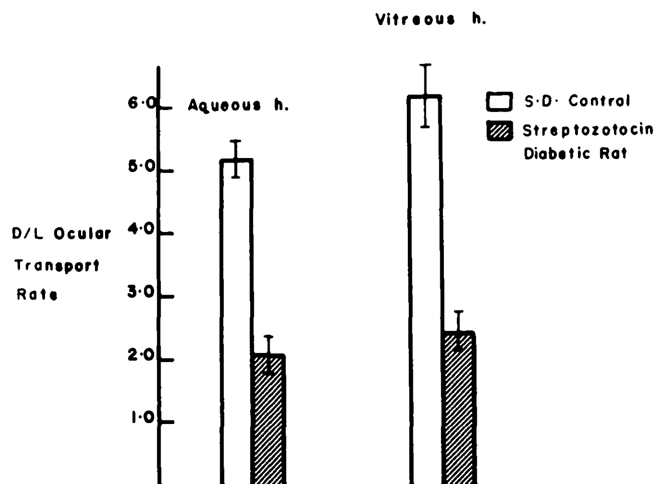
DISCUSSION

The present study clearly demonstrates that long-standing diabetes mellitus, induced in rats with streptozotocin, results in changes in glucose transport into the eye compartments. It is seen that L-glucose, which moves passively from the blood to the eye fluids, enters into both the aqueous and vitreous humors at a significantly faster rate than in normal animals, suggesting a general increase in passive permeability. Recent studies using fluorescein have revealed that the diabetic rat displays a greater leakage of dye into ocular fluids.[5,6] The present findings provide further evidence that such an increase in permeability occurs for glucose.

The finding that the overall D-glucose transport constants as estimated with [^{3}H]-3-O-methyl-D-glucose were diminished by about 30% across the plasma-aqueous and plasma-vitreous barriers does not necessarily mean that unidirectional rates of bulk glucose movement are decreased. This is reasoned from a previous study in normal rats given plasma glucose to produce a similar hyperglycemia.[2] In these normal rats, D-glucose rate constants were decreased by 60% and 53% across the aqueous and vitreous barriers, respectively, at glucose levels comparable to those encountered in the diabetic rat (470 mg/dl). This is presumably due to saturation of the carrier transport. The fact that in the diabetic rat, transport constants did not decrease to these same levels may be attributed to an increase in passive movement of glucose. Thus, the combination of the decreased rate constants as observed in normal hyperglycemic rats and the increased passive rate constants observed in the diabetic rat yield estimated rate constants of 0.046 and 0.024 min^{-1} across the aqueous and vitreous barriers, respectively, thus similar to values reported in Table 1. Consequently, we conclude that in the long-term diabetic animal, there is no demonstrable change in the kinetics of the ocular glucose carrier systems.

The finding that glucose transport was altered to a similar extent in passage from plasma to aqueous humor and from plasma to vitreous humor warrants further comment. The principal transport barriers to the ocular fluids are distinct and separate, namely, the retinal pigmented epithelium and the ciliary epithelium, respectively. The capillaries perfusing these epithelial barriers are fenestrated, which presumably allows for unimpeded passive movement of glucose. Thus, it is reasonable to assume that the increased passive movement of glucose across the plasma ocular barriers would occur at these tight-junction-containing layers, rather than at the capillaries. The similar values for passive transport change at both barriers may be coincidental, but it may also reflect some common defect due to the diabetic state which affects the barrier to passive movement of glucose at a variety of sites. The mechanism of this defect remains to be determined. However, in the presence of hyperglycemia such increased permeability would greatly increase the movement of glucose into tissues in which glucose transport is not dependent on insulin. Such increased passive movement of glucose may thus be a contributing factor to the complications of diabetes. The glucose-transport-facilitating carrier route does not appear to be affected by the insulin deficiency of the diabetic rat as had been previously suggested,[7] yet passive movement is increased. Whether the increased passive transport across ocular barriers of the diabetic rat is related to the lack of insulin or the hyperglycemia or other unknown factors of the diabetic state remains to be investigated.

FIGURE 1. A comparison of the 3-O-methyl-D-glucose/L-glucose transport ratio for control and long-term streptozotocin-diabetic rats in both the aqueous and vitreous humors. The values are reported as mean ± SD. The standard deviations for the ratios were estimated as the normalized geometric mean of the deviations for the 3-O-methyl-D-glucose and L-glucose transport rates.



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