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Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus

(glucose metabolism/streptozotocin/brain capillary metabolism/endothelial cells)

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ABSTRACT We used the Oldendorf brain uptake index method to study the blood–brain barrier transport of several metabolic substrates in diabetes. Glucose transport into the brain was decreased by 1/3 in rats with moderate diabetes induced by prior injection of streptozotocin (65 mg/kg of body weight). The transports of mannose and the poorly metabolized hexoses 2-deoxyglucose and 3-*O*-methylglucose were similarly reduced. Likewise, brain glucose transport was decreased in rats with alloxan-induced diabetes. These alterations in brain hexose influx appeared to be related to chronic (1–2 days) hyperglycemia rather than to insulin-lack *per se*. Thus, starvation of the diabetic rats for 48 hr restored both the plasma glucose concentration and brain hexose transport to normal. Conversely, the substitution of 10% sucrose for their drinking water both increased plasma glucose and decreased hexose transport in insulin-treated diabetic rats. The 45% decrease in maximal glucose transport rate observed and the uniformity of diminished hexose transport probably imply a decrease in the number of available high-affinity transport carriers at the blood–brain barrier. This defect was specific for hexoses in that the transports of neutral and basic amino acids and of β -hydroxybutyrate were not similarly affected. These results suggest that chronic hyperglycemia decreases the number of hexose carrier molecules available at the blood–brain barrier. Such an adaptation could operate to decrease the net flux of glucose into the brain during sustained hyperglycemia. It also may explain the abnormal sensitivity to abrupt blood glucose lowering in patients with diabetes mellitus.

Endothelial cells in brain capillaries constitute the blood–brain barrier (1–5). As such, they directly determine the transport of substrates into the brain (6), and they indirectly regulate the use of these substrates for such processes as fuel metabolism and neurotransmitter synthesis (7). Although the metabolism of glucose (and probably of other substrates) may be altered within brains of rats with insulinopenic diabetes (8), the effects of diabetes on its blood–brain barrier transport remain largely undefined. To examine this question, we have compared the transports of hexoses and other substrates across the blood–brain barrier in normal and diabetic rats, using the brain uptake index (BUI) of Oldendorf (9).

This report describes the effect of streptozotocin-induced diabetes of varying severity and duration on the transports of (i) glucose, (ii) two nonmetabolized hexoses (3-*O*-methylglucose and 2-deoxyglucose) that use the same blood–brain barrier transport system as D-glucose does, (iii) β -hydroxybutyrate, (iv) tyrosine, (v) tryptophan, and (vi) lysine. The results indicate that hexose transport into the brain is depressed in diabetic rats and is due to chronic hyperglycemia. Similar transport changes do

not occur with other substrates. A preliminary communication describing aspects of this work has appeared (10).

MATERIALS AND METHODS

Male Sprague–Dawley rats (150–250 g) were housed in pairs in hanging cages and given ad lib access to Purina laboratory chow except as described. Animals were exposed to light between 8 a.m. and 8 p.m. daily.

Diabetes mellitus was induced by an intracardiac injection of streptozotocin (11) (65 mg/kg of body weight; provided by the Upjohn Company or purchased from Sigma) or alloxan monohydrate (40 mg/kg of body weight), administered after an overnight fast. The existence of diabetes in individual rats was confirmed by the presence of fasting glycosuria and hyperglycemia.

All radionuclides were obtained from New England Nuclear. Test compounds were labeled with ¹⁴C and their BUIs were measured with reference to concurrently injected ³H₂O. ^{113m}In was obtained by elution from a ¹¹³Sn generator (Commissariat à l'Énergie Atomique, Sorin, France) with 0.01 M HCl. Radionuclides utilized included ³H₂O (NET-001B, 1 mCi/g; 1 Ci = 3.7 × 10¹⁰ becquerels), L-[U-¹⁴C]tyrosine (NEC-289, 380 mCi/mmol), L-[*side chain*-3-¹⁴C]tryptophan (NEC-367, 50 mCi/mmol), L-[U-¹⁴C]lysine (NEC-280, 270 mCi/mmol), 3-*O*-[*methyl*-¹⁴C]methyl-D-glucose (NEC-377, 20 mCi/mmol), D(-)- β -[3-¹⁴C]hydroxybutyric acid, potassium salt (NEC-637, 10 mCi/mmol), D-[U-¹⁴C]glucose (NEC-042X, 200 mCi/mmol), 2-deoxy-D-[U-¹⁴C]glucose (NEC-720, 300 mCi/mmol), and *n*-[1-¹⁴C]butanol (NEC-130, 0.5 mCi/mmol). Rats were anesthetized with pentobarbital (45 mg/kg of body weight; Nembutal, Abbott) in the early afternoon, and the BUI for any particular compound was determined soon thereafter by using the single-injection technique of Oldendorf (1, 12). BUI values for all test compounds were multiplied by a correction factor (0.86) to account for incomplete penetration of ³H₂O into the brain 5 sec after intracarotid injection. The BUI for β -hydroxybutyrate was performed by using ^{113m}In chelated to EDTA as a marker for the intravascular space. This corrects for residual radioactivity in the vasculature of the brain at the time of sacrifice—i.e., 5 sec after injection (12). Kinetic determinations and estimations of cerebral blood flow and brain–blood barrier symmetry were performed essentially as described by Pardridge and Oldendorf (13). The computations differed in that kinetic constants were directly derived from the nonlinearized data by using a least-

Abbreviation: BUI, brain uptake index.

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squares method of curve fitting for nonlinear regression analysis (BMDP 3R; University of California, Los Angeles).

Statistical analyses were performed by using Student's *t* test and one-way analysis of variance.

RESULTS

Characterization of the Model. Rats injected with streptozotocin developed diabetes mellitus within 24 hr as judged by the presence of fasting glycosuria and a fasting blood glucose level of 21.6 ± 2.1 mM versus 8.6 ± 0.4 mM for controls. Because the more severely diabetic rats had a high mortality rate when not treated with insulin, they were used preferentially within the first few days after streptozotocin injection. Such animals were polyuric (123 ± 7 ml/day) and had heavy glycosuria (9.2 ± 0.2 g/day). Insulin-treated diabetic rats had less polyuria (17 ± 6 ml/day) and glycosuria (0.5 ± 0.2 g/day), whereas intermediate values (63 ± 22 ml/day and 2.7 ± 0.8 g/day) were noted in insulin-treated diabetic rats that had had 10% sucrose added to their drinking water.

Effects of Diabetes and Insulin on Blood-Brain Barrier Glucose Transport. Among rats with untreated diabetes, [^{14}C]glucose transport into the brain was diminished by 33% ($P < 0.05$) (Table 1). The addition of 2 units of insulin to the 200-ml injection bolus, yielding a final bolus insulin concentration of 10 units/ml, failed to influence glucose transport. In contrast, treatment of the streptozotocin-induced diabetic rats with protamine zinc insulin for 1 wk prior to the study (7.5 units/kg of body weight, administered at 2 p.m.) tended to restore glucose transport to normal. The transport of mannose into the brain showed a similar response to both diabetes and insulin.

The decrease in blood-brain barrier hexose transport among diabetic rats was evident within 24 hr of streptozotocin administration and persisted for as long as the animals were studied (i.e., up to 25 days).

Transports of Other Hexoses. Intracellular glucose metabolism may be altered in brains of rats with experimentally in-

duced diabetes (10). To see if this could account for the observed decrease in its extraction, we also assessed the transport of the poorly metabolized hexose 2-deoxy-D-glucose and that of 3-O-methyl-D-glucose, which is not metabolized at all in mammalian tissues. Both of these hexoses have been shown to have affinities for the blood-brain barrier hexose carrier similar to that of glucose (14). The transport of both of these sugars, like that of glucose, was significantly depressed in diabetic rats (Table 2).

Relationship of Hexose Transport to Ambient Glucose Concentration. The relationship of the decrease in hexose transport in the diabetic rats to the degree of hyperglycemia was suggested by finding that it was most marked among the rats with the largest glycosuria (data not shown). Also glucose transport tended to be depressed in insulin-treated diabetic rats despite insulin treatment when they were fed sucrose for 3 days to make them hyperglycemic (Table 1).

To test this relationship in a further and more direct manner, rats made diabetic with streptozotocin (65 mg/kg) were starved for 48 hr prior to use to lower their blood glucose, and their transport of 3-O-methylglucose into the brain was compared with that of fed diabetic rats. Serum glucose concentration in these rats at the time of the study averaged 115 mg/dl versus 525 mg/dl for a comparable group of diabetic rats fed ad lib. The extraction of 3-O-methylglucose by the brain of these rats was $36 \pm 2.2\%$ versus $23 \pm 1.8\%$ for the fed group (Table 2).

As further evidence that only chronic changes in blood glucose concentrations change its brain transport, similar alterations in glucose transport were not observed when the blood glucose concentration was acutely altered. Thus, injection of control animals with an intravascular bolus of dextrose (1 g/kg of body weight) that raised blood glucose levels from 145 to 279 mg/dl did not affect brain glucose transport 10 min later. Likewise, glucose transport was unaltered in 92-g control rats injected 1 hr previously with sufficient crystalline zinc insulin (54 units/kg of body weight administered intravascularly) to produce hypoglycemia (23 mg/dl).

Alloxan Diabetes. The decrease in hexose transport in the diabetic rats was not due to a direct effect of streptozotocin. This is indicated by the finding that blood-brain barrier glucose transport was similar in rats made diabetic with alloxan monohydrate (40 mg/kg of body weight given intravascularly 4 days previously; Sigma) (Table 3). Although streptozotocin contains a glucose moiety attached to a nitrosurea, this compound apparently has very little affinity for the blood-brain barrier hexose carrier because addition of an enormous quantity of this drug

Table 1. Effect of diabetes on hexose transport across the blood-brain barrier

Test compound	Rats		% extraction*
	n	Treatment	
Glucose	16	Control	39.6 ± 3.1
Glucose	16	Diabetic	$26.6 \pm 1.4^\dagger$
Glucose	5	Diabetic, insulin-treated	37.6 ± 2.3
Glucose	5	Diabetic, insulin-treated and sucrose-fed	$31.4 \pm 4.4^\dagger$
Glucose with Insulin	6	Diabetic	26.4 ± 4.1
Mannose	6	Control	20.3 ± 1.4
Mannose	6	Diabetic	$16.2 \pm 1.2^\dagger$
Mannose	6	Diabetic, insulin-treated	19.3 ± 0.9

Animals were anesthetized with pentobarbital, and then 0.25 μCi of ^{14}C -labeled test compound and 1 μCi of $^3\text{H}_2\text{O}$ in buffered Ringer's solution were injected rapidly into the common carotid artery. Diabetes was induced 3-11 days earlier with streptozotocin (65 mg/kg of body weight). Insulin-treated animals received 7.5 units of protamine zinc insulin per kg of body weight daily for 1 wk prior to use. Sucrose-fed animals were given 10% sucrose as drinking solution ad lib in addition to laboratory chow. Where glucose and insulin are listed together, [^{14}C]glucose was injected together with 2 units of crystalline zinc insulin in the injection vehicle.

* Mean \pm SEM.

$^\dagger P < 0.05$ compared with control values.

Table 2. Effect of diabetes on transport of glucose analogs across the blood-brain barrier

Test compound	Rats		
	Treatment	n	% extraction*
2-dGlc	Control	7	44.3 ± 2.2
2-dGlc	Diabetic	9	$36.8 \pm 1.3^\dagger$
3-MeGlc	Control	8	26.2 ± 1.9
3-MeGlc	Diabetic	9	$20.2 \pm 1.9^\dagger$
3-MeGlc	Diabetic	6	23.3 ± 1.8
3-MeGlc	Starved diabetic	6	36.3 ± 2.2

The animals were anesthetized with pentobarbital, and a BUI was determined for 2-deoxy-D- [^{14}C]glucose (2-dGlc) and for 3-O-[methyl- ^{14}C]methyl-D-glucose (3-MeGlc) in control and diabetic rats [streptozotocin was administered (65 mg/kg of body weight) 4-7 days earlier]. All animals were fed ad lib on laboratory chow except the last group, which had all food withheld for 48 hr prior to use in the experiment. This group, like all others, was given free access to drinking water to maintain adequate hydration.

* Mean \pm SEM.

$^\dagger P < 0.05$ compared with control values.

Table 3. Effect of alloxan diabetes on brain glucose extraction*

Test compound	Rats		% extraction*
	Treatment	n	
Glucose	Control	6	40.5 ± 2.8
Glucose	Alloxan	5	32.3 ± 1.5 [†]

The brain extraction 5 sec after injection of D-[¹⁴C]glucose was measured with reference to ³H₂O with correction for labeled water's incomplete (86%) brain penetration. Alloxan monohydrate (40 mg/kg of body weight) was given intravenously 4 days prior to experimentation and produced diabetes in all animals in the second group.

* Mean ± SEM.

[†] P < 0.05 compared with control values.

(100 mg/ml) to the labeled injection solution diminished glucose transport by only 14%. This drug concentration is probably several orders of magnitude above those even transiently achieved in the bloodstream after diabetes-inducing doses, thus making it very unlikely that streptozotocin binding to the hexose carrier would be responsible for the diminished hexose transport in streptozotocin-treated animals.

Kinetic Studies. To determine the affinity constants (K_t) and maximal rates of glucose transport (T_{max}) in control and diabetic rats, various concentrations of unlabeled D-glucose were added to the injection bolus, and the brain's extraction of labeled glucose relative to ³H₂O was determined. The results suggest that the decreased extraction of glucose in the diabetic rats is due to a decrease in transport capacity (Fig. 1). The T_{max} for glucose transport was decreased by 45% in the diabetic rats, indicating a decrease in the number of hexose transport carriers at the blood-brain barrier luminal surface. In contrast, the K_t for glucose decreased slightly, suggesting an increase in the affinity of the carriers for glucose. If anything, this would have enhanced glucose extraction.

Capillary Leakiness and Cerebral Blood Flow. Capillary leakiness has been described in diabetics, especially in retinal capillaries. Because such leakiness conceivably will render ³H₂O extraction inappropriate as a standard for assessing blood-brain barrier glucose uptake, we measured both the absolute extraction of ³H₂O and its extraction by brains of diabetic and control rats. The BUIs of ³H₂O, expressed by using [¹⁴C]butanol as a reference standard, did not differ among diabetic and control rats (Table 4).

The absolute extraction of tritiated water (equal to its recovery in the total brain tissue) was compared in diabetic and control rats at 5 sec, 2 min, and 4 min after injection. At no time did the absolute extractions differ significantly among the two groups (Fig. 2 *Upper* and *Lower*). A rate constant for water efflux can be estimated from these data by calculating the slope of the line derived by plotting the logarithm of absolute extraction of water against time after injection. By assuming a volume of distribution (V_d) of water in rat brain of 0.78 mg/g, and an initial extraction of 0.85 (Table 4), one can estimate cerebral blood flow. By using efflux constants of 0.423 for control and 0.393 for diabetic rats, cerebral blood flow can be estimated to be 0.38 and 0.36 mg/g per min in control and diabetic rats, respectively. These values are lower than would be found in unanesthetized animals and probably reflect the effects of anesthesia. They also indicate that the differences in blood-brain barrier hexose extraction observed in diabetic rats are not explained by altered cerebral blood flow.

Bidirectionality of Hexose Transport. By calculating the absolute extraction (recovery) for 3-O-methylglucose over time, as described above for tritiated water, efflux constants for 3-O-methylglucose were obtained, equal to 0.156 for diabetic rats and 0.235 for control animals. The calculated decrease in brain

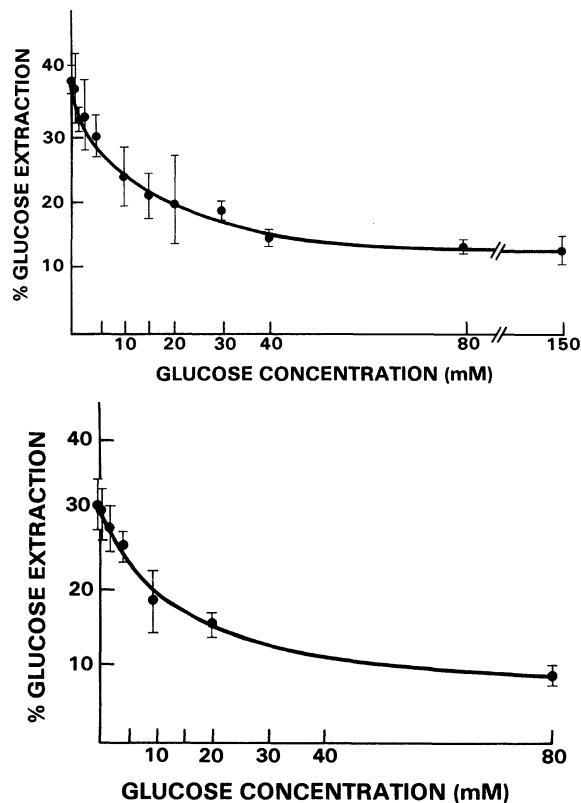


FIG. 1. Groups of 4–16 animals were prepared for intracarotid injection after administration of pentobarbital (45 mg/kg of body weight). (*Upper*) Effects in control animals of the addition of increasing amounts of unlabeled D-glucose on D-[¹⁴C]glucose brain transport from a bolus injected into a common carotid with ³H₂O used as an internal reference. Values are expressed as the percentage of extraction after correction for incomplete labeled water penetration (0.86) into the brain. (*Lower*) Data for animals made diabetic by administration of streptozotocin (65 mg/kg) 1 wk earlier. Calculated kinetic parameters of transport: K_t , 11.5 mM (*Upper*) and 9.1 mM (*Lower*); T_{max} , 1.8 μ mol/g per min (*Upper*) and 1.0 μ mol/g/min (*Lower*).

3-O-methylglucose efflux would be 33.6% in the diabetic rats. However, the slight decrease in water efflux among diabetic animals noted previously would cause an overestimation of 7.1% of the 3-O-methylglucose efflux. Correcting for this, the rate of brain 3-O-methylglucose efflux in diabetic animals appears to be 26.5% less than controls. This is roughly comparable to the 22.9% decrease in influx (Table 2). The symmetry of the blood-brain barrier in control animals can be examined by comparing influx and efflux. The efflux of 3-O-methylglucose among normal rats is calculated to be 26.4%, whereas the observed influx was measured to be 25.6%. Influx for diabetic animals was 20.1%, whereas the efflux calculated from the above data was 17.6%. These observations suggest that the

Table 4. Effect of streptozotocin diabetes on tritiated water extraction by brain

Rats		
Treatment	n	% extraction*
Control	7	85.9 ± 3.8
Diabetic	7	89.3 ± 6.1

The brain extraction of ³H₂O at 5 sec was measured in control and streptozotocin-induced (65 mg/kg of body weight 3 days previously) diabetic rats in reference to *n*-[¹⁴C]butanol. It was assumed that butanol completely penetrated the blood-brain barrier in the time period. * Mean ± SEM.

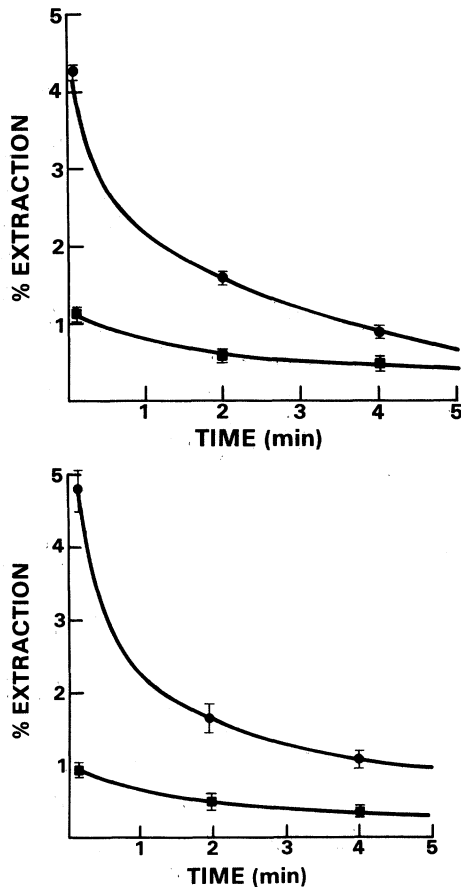


FIG. 2. Groups of 4–6 animals were anesthetized with pentobarbital (45 mg/kg of body weight) intraperitoneally. Each animal received an injection of 0.25 μ Ci of 3-O-[methyl- 14 C]methyl-D-glucose (■) and 1 μ Ci of 3 H $_2$ O (●) at time 0 into the left common carotid artery. (Upper) Recovery of each of these radionuclides in control animals' ipsilateral forebrains at 5 sec, 2 min, and 4 min. (Lower) Similar data for animals made diabetic with streptozotocin (65 mg/kg of body weight) 5 days earlier.

blood–brain barrier handling of glucose is bidirectionally symmetric in both diabetic and control rats, in keeping with the observations of others (13).

Amino Acid and Ketone-Body Transport. The transport of amino acids between blood and brain utilizes carrier molecules that are distinct from those transporting hexoses (14). We found blood–brain barrier transport of tyrosine and tryptophan, both of which enter the brain by the neutral amino acid carrier (12), were not altered in diabetic rats (Table 5). Similarly the transport of the essential amino acid lysine, which enters the brain

Table 5. Amino acid blood–brain barrier transport in diabetes

Test compound	Rats		% extraction*
	Treatment	n	
Tyrosine	Control	6	41.8 \pm 3.0
Tyrosine	Diabetic	6	45.6 \pm 4.1
Tryptophan	Control	11	33.8 \pm 4.5
Tryptophan	Diabetic	12	26.1 \pm 2.8
Lysine	Control	5	14.3 \pm 1.2
Lysine	Diabetic	4	13.7 \pm 1.1

The percentage of brain extraction of the 14 C-labeled test compound was measured in reference to 3 H $_2$ O penetration into the ipsilateral hemisphere after carotid injection. No significant differences between diabetic and control rats were found.

* Mean \pm SEM.

Table 6. Effects of diabetes and insulin treatment on the blood–brain barrier transport of hydroxybutyrate

Test compound	Rats		% extraction*
	Treatment	n	
β -OHBut	Control	6	5.1 \pm 0.6
β -OHBut	Diabetic	5	2.5 \pm 0.3 [†]
β -OHBut	Insulin-treated diabetic	6	8.0 \pm 0.9 [†]

The brain extraction of β -[14 C]hydroxybutyrate (β -OHBut) was measured with reference to 3 H $_2$ O; each sample was corrected for radioactivity remaining within the blood vessels by using 113m In chelated to EDTA as a vascular space marker. Diabetes was induced with streptozotocin (65 mg/kg of body weight) 4–11 days earlier. Insulin treatment of diabetes was with protamine zinc insulin (7.5 units/kg of body weight) for 6 days.

* Mean \pm SEM.

[†] $P < 0.05$ compared with control values.

by a basic amino acid carrier, was also normal. The BUI of β -hydroxybutyrate was increased in diabetic rats to 8.0 \pm 0.9% (Table 6). The blood β -hydroxybutyrate concentration of these animals was 2.8 \pm 1.2 mM. By contrast, control animals, whose blood β -hydroxybutyrate concentration was 0.12 \pm 0.02 mM, had a BUI for β -hydroxybutyrate of 5.1 \pm 0.6%. Interestingly, 6 days of insulin treatment of diabetic rats (7.5 units/kg of body weight of protamine zinc insulin; group shown at the bottom of Table 6) decreased the BUI for β -hydroxybutyrate to values significantly below those of both control and untreated diabetic animals. Although blood β -hydroxybutyrate concentrations were not measured in this insulin-treated diabetic group, two each among both control and untreated diabetic animals exhibited qualitative ketonuria measurable by urine Acetest (Ames, Elkhart, IN) testing.

DISCUSSION

These results indicate that experimentally induced diabetes specifically diminishes hexose transport into the brain. The transport defect was observed in rats made diabetic with streptozotocin and with alloxan. It was substantially corrected by prolonged treatment with insulin *in vivo*, but it was not acutely altered by insulin therapy. In addition to D-glucose and mannose, the transport of the glucose analogs 2-deoxy-D-glucose and 3-O-methylglucose was depressed, suggesting that the transport defect is not due to impaired metabolism of glucose within the brain (8).

Several lines of evidence implicate chronic hyperglycemia, rather than lack of insulin, as the proximate cause of diminished brain hexose transport in diabetes. First, one to several days of hyperglycemia were required for decreased transport to be manifest in diabetic rats. Acute alterations in blood glucose for minutes to hours produced no change. Second, in rats treated with insulin, the transport defect was more marked when diabetic control was poor. Thus, the provision of insulin had little effect if hyperglycemia was maintained, as in the group given 10% sucrose as their drinking solution. Third and most important, chronic normalization of blood glucose by starvation rectified hexose transport into the brain even though plasma insulin concentrations, if anything, would have been diminished further in these animals.

The exact mechanism for the decrease in glucose transport across the blood–brain barrier of diabetic rats remains incompletely explored. The 45% diminution in maximal glucose transport capacity suggests that it might be related to a decrease in the number of available hexose carriers at the luminal surface of brain capillaries. The finding of a similar decrease in the transport of other hexoses is consistent with this notion. One

can only speculate as to how chronic hyperglycemia produces this phenomenon. Perhaps the glucose transport proteins contain multiple glycosylation sites as in erythrocytes (see ref. 15), and their increased glycosylation by exposure to high glucose concentrations diminishes their availability or effectiveness. Another explanation is suggested by observations on glucose transport by fat cells (16) and muscle (17). In both of these tissues, glucose carrier proteins may be recycled between cell surface membranes and intracellular organelles, and insulin favors their appearance at the cell surface. Whether such an arrangement exists in brain capillary endothelial cells and, if so, whether the glucose transporters are sequestered intracellularly in diabetes remains to be determined.

Our data suggest that the diminished brain hexose transport in diabetes is specific. Transports of neutral amino acid (tyrosine and tryptophan) and basic amino acid (lysine) into the brain were not affected, whereas the transport of β -hydroxybutyrate was increased. Alterations in capillary leakiness and cerebral blood flow were not detected.

Altered brain glucose transport in diabetes may have several physiological ramifications. DeFronzo *et al.* (18) have shown that such sequelae of hypoglycemia as adrenergic activation and counterregulatory hormone secretion occur at higher blood glucose levels in diabetic than in nondiabetic humans (19). Our observations suggest that this enhanced susceptibility to hypoglycemia may result from a decrease in the availability of circulating glucose to the brain, secondary to its impaired transport across the blood-brain barrier. Insulin also acutely decreased the blood concentrations and the brain transport of alternative fuels for the brain, such as β -hydroxybutyrate, and thus may enhance this susceptibility.

Decreased brain glucose transport may exert a protective effect by reducing the osmotic load (net glucose flux) entering the brain in the presence of chronic hyperglycemia. The decreased transport coupled with the previously described decrease in glucose metabolism (8) by the brain could represent a synergistic adaptation in metabolic fuel handling.

Our findings suggest that specific transport defects into the brain occur in diabetes mellitus. Similar alterations in transport have been described in portocaval anastomosis, in starvation, during development, and as a result of the administration of drugs such as lithium (20–23). To what extent alterations in glucose transport by brain endothelial cells have important consequences for their functioning or for the functioning of the brain itself needs to be explored.

The finding of decreased uptake of glucose, enhanced β -hydroxybutyrate uptake, and an unchanged uptake of various amino acids across the blood-brain barrier in uncontrolled diabetes may reflect the operation of intracellular feedback mechanism akin to those for enzymes. Perhaps chronic hyperglycemia provides a signal that slows the formation or accelerates the catabolism of the blood-brain transport macromolecules. It would be interesting to determine whether the synthesis or turnover of these macromolecules is altered and, if so, what the

mechanisms are that couple these processes to the ambient glucose concentration.

While this manuscript was in preparation, Gjedde and Crone (24) also described a suppression of glucose transport into the brain in diabetic rats. Although their study used a different methodology from ours and yielded different kinetic parameters for glucose transport, the effects described are qualitatively similar.

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