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Glucose Transfer into Rat Brain During Acute and Chronic Hyperglycemia

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Chronic hyperglycemia has been reported to decrease the maximum velocity of glucose transport across the blood–brain barrier by 30 to 40%. However, available measurements of brain glucose content during chronic hyperglycemia are consistent with an unaltered transport system. Because of this discrepancy the brain capillary permeability–surface area product (PA) was measured in awake-restrained rats during acute and chronic hyperglycemia. Acute hyperglycemia was produced by intraperitoneal injection of glucose, and chronic hyperglycemia was produced by treatment with streptozotocin. PA was measured using an intravenous tracer method. PA decreased during hyperglycemia, consistent with saturation kinetics for transfer. However, PA was similar in acutely and chronically hyperglycemic rats. These data suggest that down-regulation of facilitated glucose transport into the brain does not occur during chronic hyperglycemia.

KEY WORDS: glucose transport; blood–brain barrier; hyperglycemia; streptozotocin; diabetes.

INTRODUCTION

The movement of glucose across the blood–brain barrier displays saturation kinetics consistent with the presence of a carrier-mediated transfer mechanism (Crone, 1965). The kinetics of this facilitated transport is linked to the resting rate of glucose consumption (Cremer *et al.*, 1983; Hawkins *et al.*, 1983) and rapidly adapts to acute changes in the metabolic rate (Gjedde and Rasmussen, 1980; Cremer *et al.*, 1983). This system has been modeled with varying degrees of complexity (Pappenheimer and Setchell, 1973; Lund-Andersen, 1979; Gjedde and Christensen, 1984; Cunningham *et al.*, 1986), and these models allow acute changes in transport to be explained, in part, by changes in glucose concentration gradients along and

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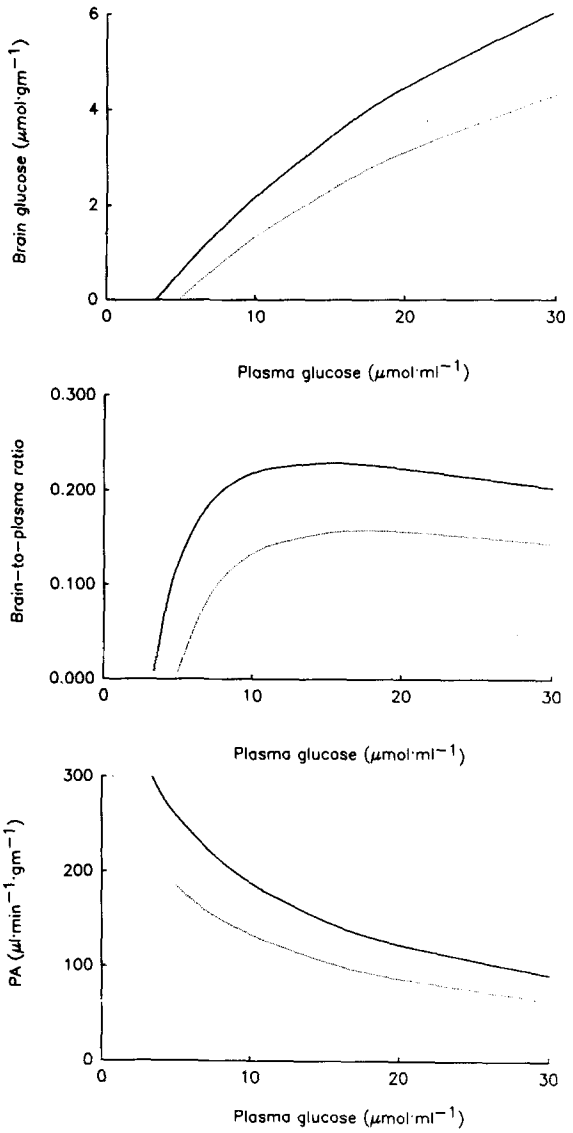

Fig. 1. Effect of varying plasma glucose on the steady-state brain glucose and the apparent PA product. Brain glucose content was calculated using a three-compartment model for glucose movement from plasma, through the endothelial cell and into brain tissue (cf. Gjedde and Christensen, 1984). A symmetric transport system following Michaelis–Menten kinetics was assumed to be present on the luminal and abluminal endothelial cell membranes and the rate of glucose utilization was $1\,\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. The solid lines were calculated using an apparent T_{max} of $3.40\,\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. The dotted lines indicate the effect of a 30% reduction in T_{max}, to $2.38\,\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. The K_t in all cases was 8 mM. The apparent PA product was calculated from the Michaelis–Menten equation.

across the capillary and by the area of capillary surface available for substrate movement (Gjedde, 1983).

Modulation of glucose transport has also been reported to occur during chronic hyperglycemia (Gjedde and Crone, 1981; McCall *et al.*, 1982) and chronic hypoglycemia (McCall *et al.*, 1986). These longer-lasting effects have been ascribed to changes in the membrane-associated transport mechanism, commonly characterized by parameters that include the maximum transport velocity (T_{max}) and the Michaelis constant (K_t). During chronic hyperglycemia suppression of the maximum transport velocity by 29 to 45% has been measured as early as 24 hr and up to 3 weeks after the induction of streptozotocin diabetes (Gjedde and Crone, 1981; McCall *et al.*, 1982).

Mathematical models of glucose transport can be used to define the relationship between the plasma glucose concentration and the brain glucose content during steady-state conditions. In the absence of a change in the distribution of glucose or its rate of utilization, a decrease in the maximum velocity of glucose transport should reduce the steady-state glucose content of the brain at any plasma glucose concentration, as shown in Fig. 1. However, direct measurements of brain glucose content during streptozotocin diabetes have not detected this decrease (Ruderman *et al.*, 1974; Blackshear and Alberti, 1974; Duckrow and Bryan, 1987). This suggests that the modulation of glucose transport during chronic hyperglycemia should be reexamined. A preliminary work has already appeared concluding that this modulation does not occur (Harik *et al.*, 1986).

A decrease in the brain capillary permeability–surface area product (PA) will reduce the maximum velocity of glucose transport measured at a constant glucose concentration. In the current work, the PA was measured during chronic hyperglycemia and was found to be the same as the PA during acute hyperglycemia of equal magnitude. This supports the contention that glucose transport activity is not reduced during chronic hyperglycemia.

MATERIALS AND METHODS

Chemicals

D-[^{14}C(U)]Glucose, 3.5–4.3 mCi/mmol, [^{3}H]inulin, 0.9–2.5 Ci/mmol, and [^{14}C(U)] sucrose, 4.6 mCi/mmol, were obtained from New England Nuclear, Boston, Mass. Soluene-350 tissue solubilizer and Dimilume-30 liquid scintillation cocktail were obtained from Packard Instrument, Downers Grove, Ill. D-Glucose and streptozotocin were obtained from Sigma Chemical, St. Louis, Mo.

Rats

Male Sprague–Dawley rats weighing between 200 and 250 g were obtained from Charles River Breeding Laboratories, Wilmington, Mass. They had free access to food and water prior to surgery.

Rat Preparation

All measurements were made on awake-restrained rats. The preparation has been described in detail (Duckrow and Bryan, 1987). Briefly, arterial and venous catheters were inserted using halothane/nitrous oxide anesthesia. Wounds were infiltrated with procaine and plaster hip casts were used for restraint. Rats were allowed at least 1 hr to recover from anesthesia and all experiments ended with rapid decapitation. Heparin (200 USP units) was injected intravenously prior to the injection of tracer compounds. Pulse rate and arterial blood pressure were monitored and arterial blood samples were obtained to measure blood gas tensions, pH, hematocrit, plasma glucose concentration, and plasma osmolality.

Chronic hyperglycemia was induced by intravenous injection of streptozotocin (60 mg/kg) 1 or 3 weeks before measurement of cerebral blood volume or glucose influx. All rats injected with streptozotocin became hyperglycemic. Acute hyperglycemia was induced by intraperitoneal injection of 2.8 *M* D-glucose (1.5 g/kg) 10 min before these measurements. Control rats received an equivalent volume of 0.15 *M* sodium chloride.

Measurement of PA

Regional PA was measured using the intravenous infusion method described by Hawkins *et al.* (1982) and Bachelard *et al.* (1973). This method uses a two-compartment model of glucose transfer from blood to brain. During short periods of tracer infusion the capillary permeability–surface area product (PA) for glucose can be estimated as

$$\mathrm{PA} = Cb^*(T) \Big/ \int Cp^*(t)\, dt \qquad (1)$$

where Cb^* is the brain tracer content at the time (T) of decapitation and Cp^* is the plasma tracer concentration. This method assumes that cerebral blood flow is very much larger than PA, and in this case there is an order of magnitude difference (Hawkins *et al.*, 1983). Flow changes induced by acute and chronic hyperglycemia are known to be similar and insufficient to invalidate this assumption (Duckrow *et al.*, 1987). As tracer accumulates in the brain there is backflux of glucose tracer from brain to blood and the use of Eq. (1) should underestimate the actual PA. However, computer simulation of glucose transfer using a two-membrane, three-compartment model (Gjedde and Christensen, 1984) indicated that this intravenous method can overestimate PA for the first 10 sec of tracer infusion. Between 10 and 20 sec of tracer exposure, the underestimate due to backflux of brain tracer is less than 4%. Accordingly, the period of tracer exposure was restricted to that interval. D-[^{14}C(U)]Glucose dissolved in 0.15 *M* sodium chloride (100 μCi/ml) was injected intravenously at a rate of 40 μl/sec. During the infusion blood drops from a femoral artery catheter were pooled at 2-sec intervals. After centrifugation 10 μl of plasma was removed for scintillation counting. Rats were decapitated at intervals from 10 to 20 sec after the onset of tracer infusion. The brain was rapidly removed from the skull and dissected. The pia-arachnoid and its vessels were removed. Samples from 17 regions were

weighed and solubilized for scintillation counting. Evaporation was minimized by transporting the brain samples in a humidified chamber. Plasma tracer concentration was integrated by the trapezoid rule. PA was calculated using Eq. (1) after correcting the amount of tracer in each regional brain sample by the amount of tracer in the blood volume of that region.

Regional blood volume was measured in 35 separate rats under conditions of normoglycemia and acute and chronic hyperglycemia to ensure an appropriate correction for intravascular tracer. A bolus of [^{3}H]inulin, 60 μCi, or [^{14}C]sucrose, 30 μCi, in 0.15 *M* sodium chloride was injected intravenously. Near the end of a 1-min equilibration period, four arterial blood samples were collected at 5-sec intervals to measure the plasma tracer content. Rats were then decapitated and regional tissue sampling was performed as above. Regional cerebral blood volume was calculated as the ratio of tracer activity in blood and brain per unit volume. Blood volume measurements were consistent with reported values (e.g., Cremer *et al.*, 1983) and did not change during acute hyperglycemia 10 min in duration or chronic hyperglycemia 1 or 3 weeks in duration. In addition, a dual-tracer method was developed to allow the simultaneous measurement of PA and cerebral blood volume in the same rat. A bolus of [^{3}H]inulin, 100 μCi, was followed 30 sec later by an infusion of [^{14}C] glucose, 10 μCi, with arterial blood sampling and decapitation as described above. PA and blood volume were compared in four rats made acutely hyperglycemic by intraperitoneal injection of glucose and four normoglycemic rats that received an equal amount of intraperitoneal mannitol. Values obtained from dual-tracer experiments were combined with data from corresponding single-tracer experimental groups.

Analytical Methods

Arterial oxygen tension, carbon dioxide tension, and pH were measured at 37°C (BMS 3 Mk 2, Radiometer Copenhagen, Westlake, Ohio). Plasma glucose was measured by an automated glucose oxidase method (glucose analyzer 2, Beckman Instruments, Fullerton, Calif.). Plasma osmolality was determined by dew-point temperature depression (Model 5500, Westcor, Logan, Utah).

Statistical Analysis

For each hyperglycemic condition (acute, 1 week, or 3 week) a concurrent normoglycemic group was prepared of equal number. The physiological variables of these six groups were compared using analysis of variance, as were their normoglycemic and hyperglycemic subpopulations. The physiological variables from each hyperglycemic condition were compared to those from the concurrent normoglycemic group using the *t* test. Regional PA during the three hyperglycemic conditions was compared using analysis of variance. Regional PAs for the two chronically hyperglycemic groups were combined and compared to that of the acutely hyperglycemic group using the *t* test.

Table I. Physiologic Variables of Rats Made Acutely Hyperglycemic by Intraperitoneal Glucose Injection or Chronically Hyperglycemic with Streptozotocin and Their Normoglycemic Controls[a]

Treatment	*N*	Weight (g)	MABP (mm Hg)	pH	P_aCO_2 (mm Hg)
Acute hyperglycemia					
Control	8	349 ± 53	127 ± 9	7.40 ± 0.04	39 ± 3
Intraperitoneal glucose	8	343 ± 41	128 ± 12	7.40 ± 0.04	39 ± 4
Chronic hyperglycemia					
Control	5	363 ± 14	133 ± 7	7.42 ± 0.03	42 ± 3
1 week after streptozotocin	4	291 ± 22[b]	129 ± 6	7.42 ± 0.02	40 ± 3
Control	6	401 ± 44	119 ± 8	7.42 ± 0.02	39 ± 3
3 weeks after streptozotocin	6	315 ± 25[b]	125 ± 9	7.42 ± 0.04	37 ± 4

Treatment	P_aO_2 (mm Hg)	Hematocrit (%)	Plasma glucose (μmol/ml)	Osmolality (mEq/liter)
Acute hyperglycemia				
Control	84 ± 10	46 ± 2	11.5 ± 1.5	290 ± 5
Intraperitoneal glucose	92 ± 9	47 ± 2	28.6 ± 4.0[b]	—
Chronic hyperglycemia				
Control	78 ± 8	46 ± 2	10.6 ± 1.6	297 ± 10
1 week after streptozotocin	80 ± 5	47 ± 1	26.8 ± 0.7[b]	312 ± 5[b]
Control	81 ± 8	45 ± 3	10.3 ± 1.0	287 ± 5
3 weeks after streptozotocin	92 ± 7[c]	46 ± 4	28.3 ± 2.3[b]	308 ± 3[b]

[a]Data are means ± SD.
[b]$P < 0.01$ compared to paired control group.
[c]$P < 0.05$ compared to paired control group.

RESULTS

Rats treated with streptozotocin failed to gain weight. The degree of hyperglycemia was the same whether acute or chronic. In other respects, the experimental groups were comparable, as shown in Table I.

The plasma glucose concentration was similar in the three normoglycemic groups, allowing regional PA values to be combined in Table II. PA was reduced during hyperglycemia, consistent with saturation kinetics for glucose transport. However, PA was the same during acute and chronic hyperglycemia 1 and 3 weeks in duration, consistent with unaltered kinetics for glucose transport. When the 1- and 3-week groups were combined and compared to the acute hyperglycemia group using the *t* test, no differences in PA were found.

DISCUSSION

The plasma glucose concentrations in the three hyperglycemic groups were the same. If the activity of the glucose transport system decreased during chronic hyperglycemia, one would expect to measure a lower PA product in those groups. No such differences were found by analysis of variance across the three groups. The 95%

Table II. Regional Capillary Permeability–Surface Area Product (PA) During Normoglycemia and Chronic Hyperglycemia of Varying Duration

Region	Normoglycemia (19)	Duration of hyperglycemia		
		10 min (8)	1 week (5)	3 weeks (6)
Motor cortex	178 ± 21	111 ± 32	100 ± 13	102 ± 7
Sensory cortex	191 ± 25	118 ± 35	109 ± 16	113 ± 19
Auditory cortex	184 ± 24	119 ± 46	99 ± 12	108 ± 13
Visual cortex	173 ± 27	112 ± 40	93 ± 11	100 ± 12
Olfactory lobe	91 ± 30	41 ± 33	28 ± 9	44 ± 15
Caudate putamen	143 ± 18	87 ± 20	76 ± 7	86 ± 12
Thalamus	160 ± 21	97 ± 29	82 ± 12	87 ± 10
Hippocampus	120 ± 18	74 ± 28	59 ± 13	71 ± 10
Hypothalamus	145 ± 28	90 ± 28	70 ± 7	75 ± 16
Superior colliculus	170 ± 25	115 ± 55	78 ± 35	99 ± 9
Inferior colliculus	209 ± 26	129 ± 44	103 ± 28	117 ± 17
Mesencephalon	147 ± 20	89 ± 25	76 ± 10	82 ± 7
Pons	147 ± 21	90 ± 23	69 ± 7	75 ± 9
Pyramidal tract	128 ± 30	85 ± 44	53 ± 11	59 ± 9
Medulla	150 ± 26	90 ± 26	72 ± 14	75 ± 9
White matter	105 ± 18	67 ± 37	50 ± 11	57 ± 9
Cerebellum	129 ± 26	83 ± 50	47 ± 11	63 ± 13

[a]The units are μl/g/min. The values are means of (N) ± SD. Regional means from hyperglycemic rats do not differ by analysis of variance.

confidence intervals for the regional PA products measured after 3 weeks of hyperglycemia indicate that an 8–17% decrease in the maximum velocity of transport (T_{max}) may have occurred and escaped detection. This analysis assumed that the Michaelis constant (K_t) remained constant at 8 mM. Although the data do not show a reduction in PA during chronic hyperglycemia, if down-regulation of glucose transport does occur, it is probably less than previously reported.

Measurements of reduced glucose transport during chronic hyperglycemia by McCall *et al.* (1982) may have resulted from use of the brain-uptake index. This method has been criticized because the tracer-containing bolus injected into the carotid artery may alter cerebral blood flow, mix with the blood during transit, or disturb the steady-state relationship between the blood and the tissue glucose concentration. Although the first two of these three effects would occur in all cases, the steady-state brain tissue glucose concentration would differ between normal and chronically hyperglycemic rats. This difference could produce the appearance of down-regulation of glucose transport. For example, Cunningham's analysis of blood–brain glucose transfer indicates that the brain-uptake index method overestimates extraction when the glucose concentration in the test bolus is higher than in the tissue (Cunningham *et al.*, 1986). This overestimate would be less in chronically hyperglycemic animals because the difference between the glucose concentration in the test bolus and that in the brain tissue is less. As a result, glucose extraction measured during chronic hyperglycemia would be lower, giving the appearance of suppression.

A similar argument cannot be applied to the method of Gjedde and Crone (1981), who also reported reduced glucose extraction during chronic hyperglycemia. Their

method allowed 1 hr of equilibration at each plasma glucose concentration and their intravenous test injection did not change the plasma glucose concentration. The parameters describing glucose transport were derived from a curve best fit to values for cerebral glucose extraction and blood flow measured over a range of plasma glucose concentrations. Cerebral blood flow did not differ in control or chronically hyperglycemic rats. However, it is not clear that the data points for cerebral glucose extraction in control rats can be distinguished from those values obtained from chronically hyperglycemic rats, as shown in the first figure in their report (Gjedde and Crone, 1981). The published data better support the conclusion that glucose transport remains unchanged.

An unchanged brain glucose transport system during chronic hyperglycemia is more compatible with previous measurements of steady-state brain glucose content. Models of glucose transport based on Michaelis–Menten kinetics (e.g., Gjedde and Christensen, 1984; Cunningham *et al.*, 1986) allow the calculation of the brain glucose content from the plasma glucose concentration, the rate of glucose consumption, and values for the parameters describing the kinetics of transport. Using the transport parameters reported by Gjedde and Crone (1981) and assuming a rate of glucose consumption of 0.5 μmol/gm/min, modulation of glucose transport by chronic hyperglycemia would result in a 25% decrease in brain glucose content when compared to acute hyperglycemia of the same magnitude. The brain-to-plasma glucose ratio would also decrease by a similar degree. As shown in Fig. 1, this ratio becomes less sensitive to small changes in plasma glucose above the normal physiologic range.

Direct measurements of brain glucose content indicate that such a decrease does not occur. Ruderman *et al.* (1974) measured a significant 15% increase in the brain-to-plasma glucose ratio after 3 days of streptozotocin diabetes. Blackshear and Alberti (1974) also studied streptozotocin diabetes in rats. After 3 days of hyperglycemia the brain-to-plasma ratio, calculated from results in their report, increased 20%. Because the individual data points were not presented, the presence of statistical significance cannot be judged. Duckrow and Bryan (1987) measured the brain-to-plasma glucose ratio after streptozotocin diabetes of 3 weeks' duration and reported a 15% increase that was not statistically significant, perhaps because of the small number of rats studied. However, the latter work also reported a decrease in the rate of glucose utilization during hyperglycemia, offering an explanation for measured increases in the brain-to-plasma glucose ratio. If there is a decrease in the maximum transport velocity for glucose entry into the brain during chronic hyperglycemia, available measurements of brain glucose content do not support the contention that this decrease may range from 29 to 45%.

Given that rapid adaptive changes in glucose influx can occur in the brain, the existence of chronic adaptive changes in the mechanism of glucose entry would be satisfying. Current concepts of the transport mechanism suggest that chronic regulation could be accomplished by changing the number of transporter complexes in the endothelial membrane or by changing the number of perfused capillaries. However, failure to measure a decrease in brain glucose conent, a decrease in PA, and, more recently, a decrease in the number of membrane-associated glucose transporters (Harik *et al.*, 1987) during chronic hyperglycemia suggests that down-regulation of glucose transport does not occur.

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