Blood-Brain Barrier Choline Transport Is Reduced in Diabetic Rats

ARSHAG D. MOORADIAN

SUMMARY

The kinetics of blood-brain barrier (BBB) choline transport in streptozocin-induced diabetic rats were compared with those of age-matched vehicle-injected control rats. The brain uptake index (BUI) of choline in diabetic rats (13.9 ± 1.1%) was significantly lower than that in control rats (22.6 ± 0.7%) ($P < .05$). This alteration in brain choline uptake appeared to occur in long-standing (9 wk) diabetes. Thus, acute hyperglycemia and diabetes mellitus for shorter periods (3 wk) did not significantly alter the BUI of choline. Insulin (8 U/kg) treatment for 5 days did not alter BUI in diabetic rats (12.9 ± 0.9%). The maximal velocity of BBB choline transport (V_{max}) in diabetic rats (0.14 ± 0.07 nmol · min^{-1} · g^{-1}) was significantly lower than the V_{max} in control rats (2.2 ± 0.8 nmol · min^{-1} · g^{-1}) ($P < .05$). The K_m of choline transport in diabetic rats (120 ± 70 μM) was modestly but not significantly lower than that in control animals (400 ± 160 μM). Similarly, the constant of the nonsaturable component of the transport (K_d) in diabetic animals (0.5 ± 0.07 μl · min^{-1} · g^{-1}) was not significantly different from that in control rats (0.9 ± 0.3 μl · min^{-1} · g^{-1}). The data indicate that diabetes mellitus in rats is associated with a decreased BBB choline transport. *Diabetes* 36:1094–97, 1987

Although angiopathic complications of diabetes mellitus are well recognized, the central nervous system (CNS) complications in general, and the effect of diabetes on cerebral capillaries in particular, have not been adequately evaluated. The down-regulation of blood-brain barrier (BBB) glucose transport in diabetes mellitus may protect the brain from the ravages of chronic hyperglycemia (1,2). Yet, albumin selectively enters the cerebral cortex within 2 wk after induction of diabetes in the rat (3). A similar increase in blood-retinal barrier permeability has been reported to occur in diabetic rats (4). In addition, a recent clinical study found impairment of memory in a group of elderly type II (non-insulin-dependent) diabetic patients (5). We have found similar alteration in streptozocin-induced diabetic (STZ-D) mice with the T-maze paradigm (6). I hypothesized that BBB-specific transport processes may be impaired in diabetes, and a possible factor contributing to the memory dysfunction in diabetic patients might be reduced blood-brain choline transport, in view of the evidence implicating the cholinergic system in memory dysfunction (7). To prove this hypothesis, I defined the kinetics of BBB choline uptake in diabetic and age-matched control rats.

MATERIALS AND METHODS

Adult male Fisher 344 rats weighing 180–220 g were used. One group of animals was made diabetic with a single intraperitoneal injection of STZ (Upjohn, Kalamazoo, MI; 45 mg/kg). Animals were selected for the studies if 1 wk after STZ injection they had urinary glucose values of 1–2% with negative to trace urinary ketone by the Ketodiastix (Ames, Elkhart, IN) and if 3 or 9 wk after STZ (the day of the experiment) the serum glucose level, measured by glucose autoanalyzer, was >300 mg/dl. Vehicle-treated control animals of similar age and sex were evaluated concurrently with the diabetic rats. In addition, a group of STZ-D animals was given protamine zinc insulin (PZI), 8 U/kg s.c. daily for the last 5 days before the experiment. The animals demonstrating serum glucose levels of 80–150 mg/dl (mean 135.2 ± 10.4 mg/dl) were selected for this study. The mean serum glucose concentration in vehicle-injected controls was 162.1 ± 8.7 mg/dl. All the animals were fed ad libitum before blood was obtained for measurement of glucose.

To evaluate the effect of acute hyperglycemia, the BUI of choline was measured in a group of rats ($n = 6$) 5 min after

From the Geriatric Research Education Clinical Center, Veterans Administration Medical Center, Sepulveda, and the Department of Medicine, School of Medicine, University of California, Los Angeles, California.
Address correspondence and reprint requests to Arshag D. Mooradian, MD, GRECC (11E), Veterans Administration Medical Center, 16111 Plummer Street, Sepulveda, CA 91343.
Received for publication 10 November 1986 and accepted in revised form 30 March 1987.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/36/10/1094/355080/36-10-1094.pdf by guest on 22 May 2022

an intravenous bolus of dextrose (1 g/kg body wt), which raised the blood glucose levels to 460.8 ± 37.9 mg/dl.

Choline transport was measured by the method of Oldendorf and Braun (8) with [^{3}H]tryptamine as the diffusible reference substance. Choline chloride (methyl-^{14}C) (sp act 52 mCi/mmol), [^{3}H]tryptamine hydrochloride (sp act 26–34 Ci/mmol), and ^3H_2O (μCi/μl) were purchased from New England Nuclear (Boston, MA). The radiochemical purity of [^{14}C]choline was >98% by paper chromatography with *n*-butanol:ethanol:acetic acid:water (8:2:1:3) solvent system, and the radiochemical purity of [^{3}H]tryptamine was 90% by thin-layer chromatography with ethanol:diethylether:ammonium hydroxide (10:10:1) solvent system.

Under pentobarbital sodium anesthesia (50 mg/kg i.p.), a total volume of 0.2 ml, containing ~0.6 μCi of [^{14}C]choline and 4 μCi of [^{3}H]tryptamine in 25 mM HEPES-buffered (pH 7.5) Ringer's saline with varying concentrations of unlabeled choline chloride, was injected into the right common carotid artery, and 15 s later the animal was decapitated. The ipsilateral forebrain was removed, the pia-arachnoid vessels were stripped away with a cotton applicator, and 0.20–0.25 g of brain tissue was solubilized in 1.5 ml Soluene (Packard, Downers Grove, IL) at 60°C for 2 h. Ten milliliters of scintillation cocktail (Instagel, Packard) were added and the radioactivity counted in a Beckman liquid scintillation counter. Counts per minute (cpm) recorded were converted to true disintegrations per minute (dpm), with appropriate corrections made for ^{14}C counts spillover in a ^{3}H window. Brain uptake index (BUI) was determined as

$$\frac{(^{14}\text{C dpm}/^{3}\text{H dpm})_{\text{brain}}}{(^{14}\text{C dpm}/^{3}\text{H dpm})_{\text{injectate}}} \times 100$$

Estimates of total velocity (V) of uptake were calculated by multiplying the BUI/100 by $E \times F \times C_a$, where $E = 0.093$ (extraction of the tryptamine reference) (9), F is blood flow in the rat forebrain, and C_a is the arterial choline concentration in the injected bolus. The F was determined as described previously (10) by $F = (B \cdot V_d)/E_w$, where B is the efflux rate constant of ^3H_2O, V_d is the volume of distribution of water (0.78 ml/g), and E_w is the fractional extraction of water from the brain by the blood (0.85). The efflux rate constant (B) was determined from the logarithmic plot of brain extraction of water at 15, 60, 120, and 240 s after intracarotid injection of ^3H_2O (Fig. 1). The control rats in this experiment were 2 mo old.

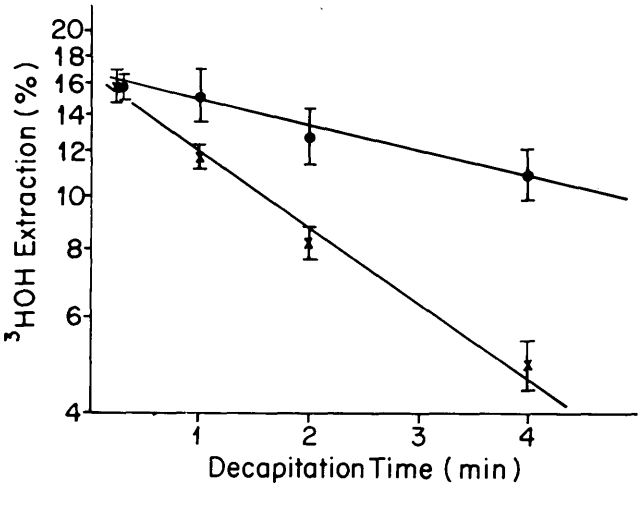

FIG. 1. Brain extraction of ^3H_2O (% of dose injected/g, means ± SE) at different time points after intracarotid injection. Only ipsilateral brain tissue was processed, as described in text. Slope of *lines* is efflux rate constant of water. *n* = 3 for each *point*. ●, Diabetic rats; X, control rats.

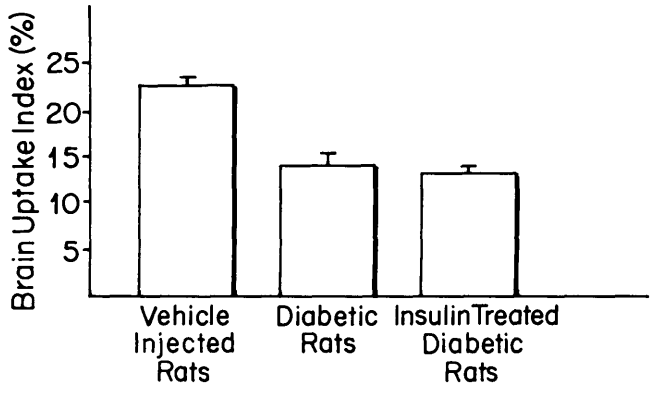

FIG. 2. Brain uptake index of choline in diabetic (*n* = 5), control (*n* = 6), and insulin-treated diabetic (*n* = 5) rats (means ± SE).

The kinetic constants of facilitated transport and passive diffusion of choline were estimated by a nonlinear regression analysis (BMDP, AR, University of California, Los Angeles) performed to fit the data to the model

$$V = \frac{V_{max} \cdot C_a}{K_m + C_a} + K_d \cdot C_a$$

where V_{max} is the maximal choline transport velocity, K_m is the choline concentration at half-maximal transport (Michaelis-Menten constant), and K_d is the diffusion constant. Statistical analysis was performed by Student's t test.

RESULTS

Figure 2 shows that the BUI of choline in diabetic rats is significantly lower than the BUI in vehicle-injected control animals [22.6 ± 0.7 vs. 13.9 ± 1.1% (mean ± SE), $P < .05$]. Insulin administration for 5 days decreased the serum glucose to 80–150 mg/dl but did not normalize the BUI (12.9 ± 0.9%). The BUI of choline in acutely hyperglycemic rats (22.3 ± 2.2%) and in rats diabetic for 3 wk only (21.8 ± 2.7%) was not significantly different from the control group (22.5 ± 1.7%). To determine if the reduced BUI of choline in diabetic rats was secondary to either increased cerebral blood flow or reduced extraction of the internal reference, the blood flow and tryptamine extraction were determined. The estimated cerebral blood flow in pentobarbital-anesthetized diabetic rats was 0.11 ml · g^{-1} · min^{-1}, which is approximately one-third of the cerebral blood flow in control rats (0.29 ml · g^{-1} · min^{-1}). The tryptamine extraction (percent of total dose injected in 1 g of ipsilateral brain tissue) in diabetic rats (5.45 ± 0.5%) was similar to that of nondiabetic control rats (5.53 ± 0.4%). Figure 3 is a plot of the total velocity (nmol · min^{-1} · g^{-1}) of BBB choline transport against various arterial concentrations of unlabeled choline. From these data the kinetic constants of BBB transport can be calculated. The V_{max} in diabetic animals (0.14 ±

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/36/10/1094/355080/36-10-1094.pdf by guest on 22 May 2022

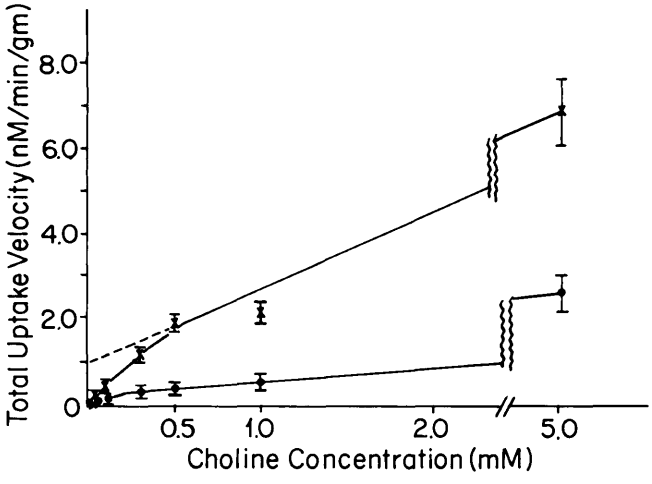

FIG. 3. Total uptake velocity of choline at different arterial concentrations of choline chloride (means ± SE). *n* = 3–6 for each *point*. X, Control rats; ●, diabetic rats.

0.07 nmol · min^{-1} · g^{-1}) was significantly lower than the V_{max} in vehicle-injected control rats (2.2 ± 0.8 nmol · min^{-1} · g^{-1}) ($P < .05$). The K_m of the transport in diabetic rats was modestly but not significantly lower than that of control rats (120 ± 70 vs. 400 ± 160 μM). Similarly, the constant of the nonsaturable transport (K_d) in diabetic animals (0.5 ± 0.07 μl · min^{-1} · g^{-1}) was not significantly different from that in control rats (0.9 ± 0.3 μl · min^{-1} · g^{-1}).

DISCUSSION

The BUI of choline in diabetic rats was significantly reduced. This alteration could not be corrected by short-term lowering of the blood glucose with insulin injections; hyperglycemia also could not alter BUI measurements, indicating that the reduced brain uptake of choline was secondary to chronic diabetic changes rather than to transient hyperglycemia. Although the cerebral vascular permeability is selectively increased in diabetic rats (3), the extraction of the internal reference [^{3}H]tryptamine was not increased in diabetic animals and, therefore, cannot account for the lowered BUI of choline. Similarly, the reduced cerebral blood flow in anesthetized diabetic rats compared with the anesthetized control rats would have falsely increased the BUI of choline in diabetic animals; the observed reduction in BUI of choline is thus actually more profound than it appears.

This alteration in brain choline uptake appears to occur only in long-standing (9 wk) diabetes, because diabetes for shorter periods did not alter choline BUI. Although the cerebral blood flow in 3-wk-diabetic rats was not determined, the animals appeared to be dehydrated. Thus, the mild impairment in BBB choline transport in these animals may have been overshadowed by reduced cerebral blood flow resulting in normal choline BUI measurements. The short-term insulin therapy did not normalize the choline BUI in diabetic rats; however, initiation of insulin therapy at the onset of diabetes might prevent the alterations in brain uptake of choline in chronic diabetes.

The kinetic constants of BBB choline transport in vehicle-injected control rats were similar to those reported previously in another strain of rats (9). However, there were significant alterations in the transport kinetics of the diabetic animals. The reduced velocity of choline transport in diabetic animals could be explained by a significant reduction in the V_{max} of transport, indicating a significant reduction in the number or mobility of choline transporters in the BBB. The K_m value was modestly but not significantly reduced in the diabetic animals, suggesting a modest increase in the affinity of the carrier to choline. This change, if anything, would have increased BBB choline transport. The molecular basis of the reducd V_{max} of choline transport in diabetic animals is not known. It could be secondary either to an absolute reduction in carrier molecules or to reduced fluidity of BBB, causing impairment in the mobility of the carrier. Reduced membrane fluidity has been recognized in some tissues from diabetic animals (11,12). We have recently found very similar kinetic changes in choline transport in aged rats (13), in which reduced membrane fluidity has also been documented (14,15). Another possibility is that choline transporter may have glycosylation sites, as is the case in the glucose-transport proteins in the erythrocytes (16), and their increased glycosylation by chronic exposure to hyperglycemia may diminish their transport capacity.

Whether the reduced BBB transport of choline contributes to the memory dysfunction in diabetes mellitus is unknown (5). Cholinergic mechanisms are implicated in memory dysfunction (7). Earlier studies have suggested that acetylcholine synthesis in the brain is limited by the availability of choline (17,18). Although somewhat controversial, it appears that exogenous choline administration enhances the synthesis of acetylcholine at least under conditions of increased cholinergic neuronal activity (19,20). Thus, the reduced BBB choline transport in diabetic animals may play a pathogenetic role in memory dysfunction.

This study indicates that specific transport defects across the BBB can occur in diabetes mellitus. Whether these defects cause specific alterations in brain metabolism that may contribute to CNS complications in diabetes remains to be seen.

ACKNOWLEDGMENTS

I thank Dr. William Oldendorf for comments and advice and Amerigo Carnizolla for technical assistance. I also appreciate the excellent secretarial services of Barbara Henderson and JoAnn Phillips.

This work was supported by the Medical Research Service of the Veterans Administration.

REFERENCES

1. McCall AC, Millington WR, Wurtman RJ: Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus. *Proc Natl Acad Sci USA* 79:5406–10, 1982
2. Gjedde A, Crone C: Blood-brain glucose transfer: repression in chronic hyperglycemia. *Science* 214:456–57, 1981
3. Stauber WT, Ong S-H, McCuskey RS: Selective extravascular escape of albumin into the cerebral cortex of the diabetic rat. *Diabetes* 30:500–503, 1981
4. Maepea O, Karlsson C, Alm A: Blood-ocular and blood-brain barrier function in streptozocin-induced diabetes in rats. *Arch Ophthalmol* 102:1366–69, 1984
5. Perlmuter LC, Hakami MK, Hodson-Harrington C, Ginsberg J, Katz J,

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/36/10/1094/355080/36-10-1094.pdf by guest on 22 May 2022

Singer DE, Nathan DM: Decreased cognitive function in aging non-insulin-dependent diabetic patients. *Am J Med* 77:1043–48, 1984
6. Flood JM, Morley JE, Mooradian AD: Learning and memory impairment in diabetic mice (Abstract). *Fed Proc* 46:1279, 1987
7. Bartus RT, Dean RL, Beer B, Lippa AS: The cholinergic hypothesis of geriatric memory dysfunction. *Science* 217:408–17, 1982
8. Oldendorf WH, Braun LD: [^{3}H]tryptamine and ^{3}H-water as diffusible internal standards for measuring brain extraction of radio-labelled substances following carotid injection. *Brain Res* 113:219–24, 1976
9. Cornford EM, Braun LD, Oldendorf WH: Carrier mediated blood-brain barrier transport of choline and certain choline analogs. *J Neurochem* 30:299–308, 1978
10. Pardridge WM, Oldendorf WH: Kinetics of blood-brain barrier transport of hexoses. *Biochim Biophys Acta* 382:377–92, 1975
11. Baba Y, Kai M, Kamada T, Setoyama S, Otsuji S: Higher levels of erythrocyte microviscosity in diabetes. *Diabetes* 28:1138–40, 1979
12. Vague P, Juhan I: Red cell deformability, platelet aggregation, and insulin action. *Diabetes* 32 (Suppl. 2):88–91, 1983
13. Mooradian AD: Blood-brain barrier (BBB) choline transport in aged rats. *Soc Neurosci Abstr* 2:1509, 1986
14. Zs-Nagy I: The role of membrane structure and function in cellular aging: a review. *Mech Ageing Dev* 9:237–46, 1979
15. Grinna LS: Changes in cell membranes during aging. *Gerontology* 23:452–64, 1977
16. Baldwin SA, Lienhard GE: Immunological identification of the human erythrocyte monosaccharide transporter. *Biochem Biophys Res Commun* 94:1401–408, 1980
17. Cohen EL, Wurtman RJ: Brain acetylcholine: increase after systemic choline administration. *Life Sci* 16:1095–102, 1975
18. Cohen EI, Wurtman RJ: Brain acetylcholine: control by dietary choline. *Science* 191:561–62, 1976
19. Trommer BA, Schmidt DE, Wecker L: Exogenous choline enhances the synthesis of acetylcholine only under conditions of increased cholinergic neuronal activity. *J Neurochem* 39:1704–709, 1982
20. Marchbanks RM, Kessler PD: The independency of choline transport and acetylcholine synthesis. *J Neurochem* 39:1424–33, 1982

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/36/10/1094/355080/36-10-1094.pdf by guest on 22 May 2022