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Blood-Brain Glucose Transfer in the Mouse

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The intracarotid injection method has been utilized to examine blood-brain barrier (BBB) glucose transport in normal mice, and after a 2-day fast. In anesthetized mice, cerebral blood flow (CBF) rates were reduced from 0.86 $ml \cdot min^{-1} \cdot gm^{-1}$ in control to 0.80 $ml \cdot min^{-1} \cdot gm^{-1}$ in fasted animals ($p > 0.05$). Brain Uptake Indices were significantly ($p < 0.05$) higher in fasted (plasma glucose = 4.7 mM) than control (plasma glucose = 6.5 mM) mice, while plasma glucose was significantly lower. The maximal velocity (V_{max}) for glucose transport was 1562 ± 303 $nmoles \cdot min^{-1} \cdot g^{-1}$, and the half-saturation constant (Km =) 6.67 ± 1.46 mM in normally fed mice. In fasted mice the Vmax was 2053 ± 393 $nmoles \cdot min^{-1} \cdot g^{-1}$ ($p > 0.05$), and the half-saturation constant (Km =) 7.40 ± 1.60 mM (not significant, $P > 0.05$). A rabbit polyclonal antiserum to a synthetic peptide encoding the 13 C-terminal amino acids of the human erythrocyte glucose transporter (GLUT-1) immunocytochemically confirmed that the mouse brain capillary endothelial glucose transporter is a GLUT-1 transporter, and immunoreactivity was similar in brain endothelia from fed and fasted animals. In conclusion, after a 2-day fast in the mouse, we saw significant reductions in forebrain weight (7%), and plasma glucose levels (27%). Increased brain glucose extraction (25%, $p < 0.05$), and a 22% increase in the unsaturated *permeability-surface area* product ($p < 0.05$) was also observed.

KEY WORDS: Blood-brain barrier glucose transporter; kinetic constants; GLUT-1 isoform immunocytochemistry; fasting; unsaturated permeability-surface area products.

INTRODUCTION

Studies of blood-brain barrier (BBB) transporters have defined eight major nutrient transporters in the rat brain (41,42). Kinetic analyses indicate that the glucose transporter (V_{max} 1-2 $umoles \cdot min^{-1} \cdot g^{-1}$) is undoubtably the most abundant BBB transporter in the rat brain. Although there are many murine genetic models of disease [such as the non-obese diabetic (NOD) mouse (35)] which might provide insight into BBB function, fewer attempts have been made to characterize BBB transporters in the mouse brain. By altering plasma glucose levels through administration of insulin or glucagon, Growdon et al. (21) estimated murine brain glucose transporter kinetics. Some more recent reports suggest BBB glucose transporter function is unchanged in hypoglycemia (10,17,45) in contrast to others suggesting an upregulation (6,12,33). In hyperglycemia due to diabetes, there are several reports that the glucose transporter is down-regulated

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(5,17,18,32,34,38), although others have found no change (13,23,31,49). Collectively, these reports suggest the need to re-examine BBB glucose transport in the mouse brain under normoglycemic conditions, and to determine if alterations in plasma glucose might modify mouse brain glucose transfer.

Therefore the present study compares BBB glucose transfer in normal and fasted (2-day) mice using the single intracarotid injection (Brain Uptake Index, = BUI) method, in conjunction with measurements of cerebral blood flow (CBF) rates. The contribution of CBF measurements is emphasized by links between flow and transport in hypoglycemic states (12) and by observations that BUI measurements increase when CBF rates decrease (37). Also, flow may be decreased in diabetes (14,28). Furthermore, a similarity between fasting and diabetes is that both are insulin-deficient states; in fasting insulin secretion is reduced (29,36) with hypoglycemia, whereas chemically induced diabetes results in an insulin deficiency and concomitant hyperglycemia.

Finally, we performed an immunocytochemical analysis using an anti-GLUT-1 antisera to identify the immunocytochemical nature of the murine BBB glucose transporter, since recent studies indicate the GLUT-3 isoform is present in canine BBB microvessels (15). Thus the objectives of the present study were (a) to define the effects of fasting upon BBB glucose transport in the mouse (kinetic characterization), in an attempt to determine a possible role of altered blood glucose levels on BBB glucose transporter function; and secondly, (b) to determine if the same glucose transporter isoform could be identified in the murine BBB glucose as rats (i.e. immunocytochemical characterization).

EXPERIMENTAL PROCEDURE

Radiochemicals. The [^{14}C]D-glucose and [^{14}C]diazepam were obtained from Amersham Australasia, Auckland, New Zealand. Tritiated water and [^{14}C]butanol were obtained from the New England Nuclear Corporation, Nuclear Supplies, Auckland, New Zealand. The tin-indium TFC3 generator was obtained from Amersham International, Buckinghamshire HP7 8NA, England. Radiochemical purity of the ^{14}C-isotopes was confirmed prior to, and after use, by thin layer chromatography on glass backed silica gel plates (Whatman LK6D, 20 × 5 cm) using a solvent system recommended by the manufacturer. Isotopic scanning was performed on a Tracemaster LB 285 linear analyzer (Berthold Analytical Instruments, Nashua, New Hampshire 03063).

Injection Solutions. A mixture was prepared containing about 0.5 uCi of [14-C] test compound (D-glucose, or diazepam), 5 μCi of ^{3}H-reference isotope (tritiated water), with or without about 50 μCi of ^{113m}In per 100 μl of buffered saline. The ^{113m}In was eluted from a tin-indium generator in dilute 0.04 M HCl, and chelated to ethylene diamine tetra-acetic acid as described previously (9). The buffer, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, HEPES, was obtained from Sigma Chemical Company (St. Louis, MO) and diluted to a final concentration of 10 mM (pH 7.55). This saline solution also contained 0-100 mM D-glucose.

Antiserum Preparation. A synthetic peptide corresponding to the thirteen amino acids at the C-terminus of the human erythrocyte glucose transporter (GLUT-1) was prepared (24) in the UCLA Peptide Synthesis Laboratory and its amino acid composition confirmed. A rabbit polyclonal antiserum to this synthetic carboxy-terminal peptide was prepared as described by Pardridge et al. (46) and designated anti-CT. The anti-CT was characterized using two techniques. The human erythrocyte glucose transporter (hGT) was purified from freshly isolated human red cells using the method of Baldwin and Lienhard (2). Western blot analysis of the hGT, using the anti-C-terminal (anti-CT) antiserum confirmed the identity of the hGT, and the ability of the anti-CT to identify glucose transporter. Secondly, absorption of the antiserum with the synthetic peptide, as described by Vinters et al. (54), eliminated antiserum reactivity with brain microvessels.

Immunostaining. Mice were decapitated, the brains removed and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C for 1-4 days, then dehydrated, and embedded in paraffin. Sections were cut at six microns, deparaffinized, hydrated and stained with anti-CT antiserum (prepared as described above). Sections were stained with anti-CT at dilutions of 1:500 to 1:1000, and corresponding controls were stained at the same dilutions with preimmune serum. The sections were hydrated, endogenous peroxidase was quenched in 0.3% hydrogen peroxide, and after rinsing in 0.01M phosphate buffered saline, blocked in 3% normal goat serum in buffer for 30 min at room temperature. The primary antiserum was applied to the sections and incubated overnight at 4°C. The sections were rinsed in buffer and the secondary antibody [goat anti-rabbit IgG] was applied for 30-min at room temperature. After a rinse in buffer, the tertiary antibody [an avidin-biotinylated peroxidase complex (ABC Elite, Vector Laboratories, Burlingame CA)] was applied for an additional 30-min at room temperature. Sections were washed with phosphate buffer, then with 0.1 M Tris buffer and incubated in cobaltous chloride for 10-min and washed again in Tris, then phosphate buffer. The sections were finally incubated in 100 ml of 0.1 M phosphate buffer containing 50 mg DAB (diamino benzedine), 40 mg ammonium chloride, 200 mg-*B*-D-glucose and 0.3 mg of glucose oxidase as described elsewhere (27). After rinsing in phosphate, the sections were either counterstained in hematoxylin, or directly dehydrated, cleared and coverslipped.

Animal Methods. Locally bred CD-1 mice of either sex (20-35g) were used throughout this study. Prior approval from the appropriate institutional review boards was obtained for these experimental studies. Mice were maintained on a bed of wood shavings with access to food and water ad libitum, unless otherwise specified. Animal quarters were maintained at 21 ± 1°C, with equal (12-hour) light and dark periods. Glucose transporter kinetics were determined from a total of 40 fed control and 38 fasted mice. A group of 8 nonobese diabetic (NOD) mice were obtained from the Auckland University colony; 4 diabetic mice had been glucosuric for at least four days, and non-glucosuric sex and age-matched mice of the same strain served as controls. Mice were weighed and anesthetized by intraperitoneal injection of sodium pentobarbital. Because mice have higher metabolic rates than rats [1], we used a dose of 60-80 mg/kg, delivered from a graduated syringe (Hamilton Company, Reno, Nevada). For brain injection studies, the animal was placed in the supine position, the left common carotid artery was isolated and separated from the vagosympathetic nerve trunk. A 25-30 μl volume of the isotopic injection solution (described above) was injected into the common carotid artery through a 30-gauge needle. It has been previously demonstrated in the rat that because the rate of injection exceeds the rate of carotid blood flow, the injection solution

traverses the brain microcirculation as a bolus with less than 5% mixing with the circulating blood (44). The mouse was decapitated 4 or 5-sec after injection and the forebrain dissected out for liquid scintillation spectroscopy. This period is sufficient for a single pass of the bolus through the brain, but short enough to minimize the efflux of labelled compound from the brain with the recirculating plasma.

Cerebral blood flow (CBF) measurements were performed on (12 control and 5 fasted) pentobarbital anesthetized mice using the artificial organ method of Van Uitert and Levy (52). An arterial (femoral) cannula filled with heparinized saline was connected to a Minipuls 2 peristaltic pump (Gilson Medical Electronics, Middleton, WI) calibrated to withdraw at the rate of 0.50 $ml \cdot min^{-1}$. Arterial withdrawal commenced simultaneously with a bolus injection of radiolabelled diazepam or butanol (about 0.1 μCi in a volume of 20 μl of normal saline) into the femoral vein. Concomitantly, the mouse was decapitated and withdrawal halted (exactly 10-sec later). Cerebral hemispheres were dissected into tared vials, and digested in Soluene (Packard Instruments, Downers Grove, IL) for scintillation counting. The arterial blood withdrawn was also completely washed into a vial, digested with a mixture of soluene:isopropanol, and decolorized by the dropwise addition of hydrogen peroxide. In some instances, excess butanol (0.1 ml) was added to the vials to prevent evaporative loss of the [^{14}C] butanol. The peroxidative reaction was forced to completion in a ultrasonic waterbath (Branson Ultrasonics, Danbury, CT) prior to scintillation counting. Blood flow rate in the tissue sampled is directly proportional to radioactivity per gram wet weight in the tissue and the radioactivity in the blood, given that the blood flow rate withdrawn into the artificial organ (0.50 $ml \cdot min^{-1}$) is known (52,53). Diazepam is completely cleared by the brain vasculature in a single transcapillary transit (50), eliminating the need to correct for possible backflux of the tracer during the experimental time period.

Date-Analyses. The brain uptake index (BUI) is a measure of extraction (E%) of the test (E_t) and reference (E_r) compounds, where $BUI = E_t/E_r - E_{Indium\text{-}/EDTA}/E_r$. As indicated previously, this is measured from the ratio:

$$BUI\ \% = \left\{\frac{[^{14}C]/[^{3}H]\text{ in brain}}{[^{14}C]/[^{3}H]\text{ injected}} - \frac{[In]/[^{3}H]\text{ in brain}}{[In]/[^{3}H]\text{ injected}}\right\} \cdot 100$$

A detailed description of indium quantitation in a beta counter is provided by Oldendorf and Szabo (39). The ^{113m}In, chelated to EDTA, does not cross intact cell membranes. Thus in the above ratio the subtrahend serves to indicate that fraction of test isotope which may remain in the vasculature, typically a very small (< 3%) fraction. These Indium Uptake Indices (the $E_{In\text{-}EDTA}/E_{[^3HOH\ reference]}$) provide an approximation of blood vascular space in the brain. In determining the relative extractions of different reference isotopes (i.e. water and diazepam), indium was not included, and relative extractions ($E_{test}/E_{reference}$) are derived directly from the minuend in the above BUI ratio.

The half-saturation constant (Km) and maximal velocity (V_{max}) of the saturable component of transport, as well as the diffusion component (K_D) of nonsaturable glucose transport were estimated as described by Pardridge (41) from nonlinear regression analyses of the extraction (E) data:

$$E = 1 - e^{-PS/F}$$

where PS, the permeability times surface area product, in $ml \cdot min^{-1} \cdot g^{-1}$ was derived as a function of the kinetic constants:

$$PS = \frac{Vmax}{Km + C} + K_D$$

and C is the injected (arterial) glucose concentration. Nonlinear regression analyses were used to derive the kinetic constants. Subroutine AR of the BMDP programs, developed at the Health Sciences Computing Facility, UCLA (Los Angeles, CA, U.S.A.) gave estimates of V_{max}, Km, K_D and PS with their respective errors. Statistically significant differences between control and treatment means and standard deviations (at the $P < 0.05$ level of confidence) were evaluated by a Student's *'t'* test. The standardized normal deviate (z-value, single tailed) was used in comparing estimates of kinetic parameters obtained from nonlinear regression analyses.

RESULTS

As indicated in Figure 1, the mouse brain capillary is highly reactive to immunohistochemically identifiable GLUT-1 epitopes. Visual inspections indicated that control mouse brain sections, prepared with the same dilutions of preimmune serum, did not react with brain capillary endothelia, nor with any part of the mouse brain. The immunoreactivity with an antiserum, corresponding to the 13 amino acids at the C-terminus of the human glucose erythrocyte transporter (Figure 1), suggests that the GLUT-1 isoform is found in the mouse BBB. There was no apparent difference in immunoreactivity of brain sections from fasted mice.

Cerebral blood flow (CBF) rates were measured in 12 barbiturate-anesthetized mice using the artificial organ technique, and the mean cerebral blood flow rate determined was $0.86 \pm 0.13\ ml \cdot min^{-1} \cdot g^{-1}$. In five fasted mice, slightly lower cerebral blood flow rates ($0.80 \pm 0.11\ ml \cdot min^{-1} \cdot g^{-1}$; $X \pm SD$; $P > 0.05$) were observed. In blood samples taken at the time of CBF measure-

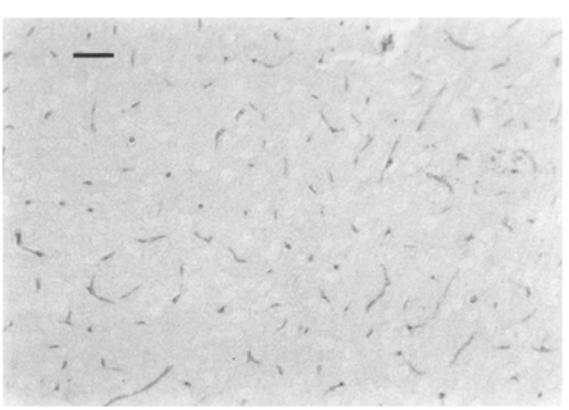

Fig. 1. Immunocytochemical localization of the brain-type glucose transporter with antiserum corresponding to the C-terminal 13 amino acids of the human erythrocyte (GLUT-1) transporter. Some reactivity was observed in the meninges (not shown) together with a strong reaction in the capillaries which comprise the blood-brain barrier. Adjacent sections from the same region of the mouse cortex stained with preimmune serum showed no reactivity with any part of the brain. Scale bar = 0.05 mm.

ments, plasma glucose levels averaged 7.4 mM in control and 5.7 mM in fasted mice. As a validation of the method, hepatic blood flow rates were determined in a number of the normal mice. Previous estimates (derived from hepatic water washout) suggest murine liver blood flow is 3.6 ± 0.3 $ml \cdot min^{-1} \cdot g^{-1}$ (8) under barbiturate anesthesia; in the present study, mean hepatic blood flow rate (± SD) was not significantly different (3.09 ± 0.75 $ml \cdot min^{-1} \cdot g^{-1}$; n = 5, CD-1 mice) using the method of Van Uitert and Levy (52). Furthermore, CBF (± SD) measured in non-glucosuric NOD (non-obese diabetic) mice measured 0.825 ± 0.279 $ml \cdot min^{-1} \cdot g^{-1}$, and 0.740 ± 0.010 ($P > 0.05$) in NOD mice which had positive glucosuric tests for 4-6 days.

Glucose transport in the mouse brain was saturable, and the measured brain uptake index decreased over the range of substrate concentrations (0.02-100 mM) examined. In mice which had been fasted two days, brain uptake indices were higher at each of the glucose concentrations examined (Figure 2). The fractional extraction of water was compared in fasted and control mice. Diazepam is known to be completely cleared by brain microcirculation in a single transcapillary passage (50) and using this as the reference compound, the E_{Water} in control (E = 72.6 ± 3.6%) and fasted (E = 78.8 ± 4.9%) mice was not significantly different ($P > 0.05$). Butanol uptake was also measured (using diazepam as the reference compound) in normal mice (E = 83.5 ± 2.7%, n = 4). As anticipated, plasma glucose levels were reduced from 6.5 mM to 4.7 mM in fasting (Table I).

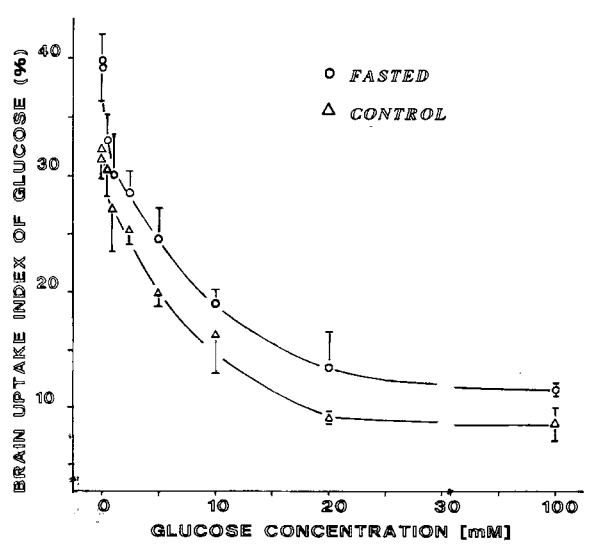

Fig. 2. A comparison of blood brain barrier uptake of glucose in control (triangles) and fasted (circles) CD-1 mice under barbiturate anesthesia. The Brain Uptake Indices depicted are means (n = 3-8), vertical bars represent ± 1SE.

Glucose Transporter Constants. Kinetic analyses of data from 40 control and 38 fasted mice (Figure 2) appear in Table 1. In control CD-1 mice, the half saturation constant for glucose transport was Km = 6.7 mM, the maximal velocity = 1.56 $nmol \cdot min^{-1} \cdot mg^{-1}$, and the diffusion component (K_D) could not be distinguished from zero. Our estimate of the BBB glucose transporter half-saturation constant in fasted mice, obtained when the data were analyzed using the two parameter model (i.e. K_D of zero), was similar. The affinity of the transporter in fasted mice (Km = 7.4 mM) was slightly, but not significantly, higher. Maximal velocity shifted in the same direction, increasing by an estimated 30% (V_{max} = 2.05 ± 0.39 $nmol \cdot min^{-1} \cdot mg^{-1}$), although this suggested increase was not significant at the 5% confidence level (Table I). In contrast, unsaturated *Permeability Surface area* products (PS = V_{max}/Km) were significantly different in control (PS = 234 ± 10 $ul \cdot min^{-1} \cdot g^{-1}$) and fasted (PS = 277 ± 13 $ul \cdot min^{-1} \cdot g^{-1}$; $p < 0.01$) mice (Table 1).

Glucose influx may be determined from the product of the plasma glucose concentration (C) and the kinetic constants, $v = (V_{max} \cdot C)/(Km + C) + K_D \cdot C$. In control mice influx $[(1.56 \times 6.67)/(13.14) + (0)] = 0.79$ $\mu mol \cdot min^{-1} \cdot g^{-1}$. Influx was not significantly changed in fasting (0.80 $\mu mol \cdot min^{-1} \cdot g^{-1}$) indicating that compensatory modulations in the BBB transporter counteract reductions in plasma glucose levels.

Body weights of fasted mice (26.4 ± 5.4 g) were reduced when compared to non-fasted controls (31.8 ± 3.3 g; $p < 0.01$) as expected. The ratio of brain:body weights was 1.08 ± 0.12% in controls and 1.24 ± 0.27% ($p < 0.05$) in fasted mice. However, brain mass was significantly reduced after a 2-day fast; cerebral

Table I. Comparison of Blood-Brain Barrier Glucose Transport in Fasted and Control Mice

Parameter	Control	Fasted
Brain Uptake Index (%)	32.3 ± 2.2	39.8 ± 2.4*
Extraction (water, %)	72.6 ± 3.6	78.8 ± 4.9
Cerebral blood flow rate	0.86 ± 0.13	0.80 ± 0.11
Km (mM)	6.67 ± 1.46	7.40 ± 1.60
Vmax (nmoles/min.g)	1562 ± 303	2053 ± 393
PS product (ml/min.g)	0.234 ± 0.009	0.277 ± 0.010*
Plasma glucose (mM)	6.47 ± 0.91	4.72 ± 0.60*
Influx	0.79	0.80

*$P < 0.05$

Standard deviations are given with Extraction, flow, and glucose levels; asymptotic standard deviations are listed for the kinetic constants.

hemispheres from control mice weighed 339.6 ± 20.3 mg (n = 11), compared to 314.9 ± 17.8 mg in fasted (n = 10) controls (P < 0.01).

DISCUSSION

Immunocytochemical data (Figure 1) demonstrate that in the mouse forebrain, antiserum to a region of the brain-type glucose transporter (GLUT-1) is specifically localized to the BBB, i.e. the microvascular endothelium. No immunoreactivity was observed in the neurons or glia. These observations are consistent with previous work establishing that the brain-type glucose transporter gene is exclusively expressed in the capillaries comprising the BBB, and not expressed in the adjacent neuropil (3). The only other site where immunoreactivity was observed was in the basal ependyma (but not apical membranes) of the mouse choroid plexus, and no differences were observed in comparing fasted and control mice. Previous studies describe how dietary alterations and starvation can produce significant changes in blood to brain transfer of nutrients (7,41). For example, ketone bodies are utilized as they appear in the circulation in fasting (40), and there is a concomitant increase in activity of the blood-brain barrier (BBB) monocarboxylic acid transporter (16,48). In contrast to the well documented alterations in transport of short chain monocarboxylic acids, conflicting reports exist regarding the effects of fasting on BBB glucose transport in the rat. Christensen et al. (6) reported that starvation causes an increase in BBB glucose transport, and this observation is supported in other studies of hypoglycemic effects (12,22,34). Crane et al. (10) found no significant changes in either phosphorylation of 2-deoxyglucose, or BBB glucose transport; consistent with studies reported no changes in glucose transporter as a result of hypoglycemia (16,45).

The significant increase in unsaturated *PS* product observed in the present study of fasted mice, may be likened to the starvation-induced increases in BBB glucose transport reported by Christensen et al. (6). Increased BUIs of glucose in fasted animals were observed in rats (10,45) and in the present study; increases in brain glucose transfer have been reported in studies reporting both upregulation (6) and no upregulation of the glucose transporter in fasting (10,45). The glucose transporter maximal velocity estimations reflect the number (density) and activity of capillary GLUT-1 transporters; V_{max} estimates are thus a more reliable and conservative indicator of altered BBB glucose transport. Our estimate of transporter maximal velocities indicate fasting causes an apparent 30% increase in this parameter, however the alteration was not significant at the 5% confidence level, consistent with the report of Crane et al. (10).

The present work suggests that in the mouse brain, one of the other changes which occurs after this brief fast is the reduction in brain mass. This was readily apparent in smaller mice (25-28 g initial body weight). The increase in brain:body weight ratios seen in fasted mice suggests reductions in body weight are comparatively greater. However, the reduction in mouse brain mass which we describe was apparently not observed in previous studies of fasted rats. It is known that ^{113m}In-EDTA is excluded by intact cell membranes, and thus the indium extraction measurements provide a comparison of brain vascular spaces. Curiously, the Extraction of Indium-EDTA was higher in fasted than control mice (E = 2.59 ± 0.48%, and 1.79 ± 0.44%, respectively). This suggests that in fasting, the vascular compartment may not be reduced as much as the brain water distribution volume. Apparently there is a relative increase in capillary density in the fasted mouse brain; increases in BUI and PS of glucose are not unexpected under such conditions.

In mice, we observed a slight reduction in CBF (Table 1); Christensen et al. (6) observed increases in CBF which were not significant, whereas Crane et al. (10) reported a decrease which was also not significant. In non-glucosuric NOD mice, CBF (0.83 $ml \cdot min^{-1} \cdot g^{-1}$) was comparable to normal CD-1 mice (Table I), but slight (10%) reductions in glucosuric NOD mice (0.74 ± 0.10 $ml \cdot min^{-1} \cdot g^{-1}$) were observed. As discussed above, in the diabetic rat model, studies of BBB glucose transport have produced conflicting reports. Recently, down-regulation of the brain capillary immunoreactive glucose transporter was seen in experimental diabetes, but no normalization of this down-regulation in streptozotocin-treated rats receiving insulin (30). This suggests a direct effect of streptozotocin on the BBB, and raises the need for alternate models for the study of BBB functions in experimental diabetes. One such model is the non-obese diabetic (NOD) mouse (35), and the present study suggests this murine diabetic model may be appropriate for further investigations of BBB function. Similarities between experimental diabetes and fasting are that both are insulin-deficient states; it is noteworthy that CBF is also reduced 20% (28) to 40% (46) in the forebrains of streptozotocin-treated diabetic rats. Curiously, when fasting is superimposed upon another treatment, such as cismethrin-induced tremors (12) or streptozotocin diabetes (47), rat CBF appears to be increased.

The use of [14-C] butanol in measurements of CBF assumes this isotope is freely cleared across the BBB, as indicated by several studies (19,49,53,55). Other studies, however, suggest butanol is not completely cleared

(20,43) and its extraction is 78-88% in barbiturate-anesthetized rats. As indicated by Crane et al. (10), this may lead to a slight underestimation of CBF when [^{14}C]butanol is used in these measurements. Diazepam is completely cleared (E = 100%) in a single transit through the brain (50) and has been utilized to estimate flow with the internal carotid artery perfusion technique (51). To ascertain the relative merits of diazepam versus butanol in flow measurements with the artificial organ technique (52) we measured CBF in the forebrains of normal mice after simultaneous injection of 14C-butanol and 3H-diazepam. Diazepam-based measurements indicated CBF = 0.86 ± 0.14 ml·min^{-1}·g^{-1}, slightly higher but not significantly different from butanol-based flow estimates (0.72 ± 0.18 ml·min^{-1}·g^{-1}). This 83% reduction in butanol-based measurements is similar in magnitude to the extraction of butanol observed in the mouse (83.5 ± 2.7%, the present study) and the rat [78-88%; (43)] brains. The CBF in barbiturate-anesthetized mice estimated in the present study (CD-1 mice = 0.86; nonglucosuric NOD mice = 0.83 ml·min^{-1}·g^{-1}) is markedly lower than the reported 2.2 ml·min^{-1}·g^{-1} (21), but presumably is attributable to their use of a different (ether) anesthetic.

Growdon et al. (21) estimated the BBB glucose transporter V_{max} = 2.1 μmol·min^{-1}·g^{-1} and the Km = 6 mM from their studies of brain glucose distribution after intraperitoneal administration of labeled glucose in ether-anesthetized mice. They noted similarities between their estimates and a previous study (employing the same method) in rats (4). The estimates of mouse BBB glucose transporter kinetics were based on a narrow range (2-25 mM) of plasma glucose levels [manipulated by insulin or glucagon administration (21)]. It is established that BBB glucose transporter activity, cerebral blood flow, and brain glucose utilization rates are linked such that an alteration in one of these parameters can produce a similar shift in the others (11,26). Given that blood flow rates were 2.5-3-fold higher in the study of Growdon et al. (21), it is not surprising that they report a higher maximal transport velocity than we estimated. The kinetic constants determined here for the normal mouse brain (Table I) indicate the saturable component of glucose transport (Km = 6.7 mM; V_{max} = 1.56 nmoles·min^{-1}·mg^{-1}) is not unlike the anesthetized rat [Km = 10 mM, V_{max} = 1-1.5 nmole·min^{-1}·mg^{-1} (10,41)].

The slight changes observed in half-saturation constants (+ 10%) and maximal velocities (+ 30%) in fasted mice, indicate that fasting-induced changes in these two parameters were in the same direction. It is remarkable that in both fed and fasted mice, glucose influx remains stable. BBB glucose transport modulates in response to reduced plasma substrate in a compensatory fashion; consequently, glucose influx is essentially unchanged in fasted mice. It has also been established that the brain of fed and fasted rodents relies on glucose (31), and very little of the total energy requirement comes from ketone bodies (25). Consequently, the brains of rats and mice may not switch to ketone body utilization in fasting as readily as other vertebrates.

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