Streptozotocin-induced diabetes progressively increases blood-brain barrier permeability in specific brain regions in rats

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The blood-brain barrier (BBB) is situated at the level of the endothelial cell and serves to partition the systemic circulation from the brain parenchyma. The BBB forms discrete microenvironments within the brain to support optimal functioning of a diverse array of neurotransmitters (1). The BBB is characterized by a well-defined basement membrane, presence of tight junctions, absence of fenestrations, and close apposition to other brain cell types, including astrocytes, pericytes, microglia, and neurons. These unique characteristics confer distinct properties that differentiate the BBB from peripheral capillaries (27). For example, tight junctions between BBB endothelial cells lead to a high transendothelial electrical resistance of 1,500–2,000 Ω·cm² as compared with 3–33 Ω·cm² in other vascular tissues (9, 13). The net result of this high electrical resistance is low paracellular diffusion and limited formation of transcapillary endocytosis, thus enabling a highly regulated and stable microenvironment within the brain. Being a dynamic barrier allows the BBB to maintain and regulate brain homeostasis and compensate for fluctuations in the systemic circulation and increased metabolic functions within the brain; however, a number of CNS-associated diseases, including human immunodeficiency virus encephalitis (58), meningitis (62), multiple sclerosis (44), Alzheimer’s and Parkinson’s diseases (3, 67), epilepsy (53), and stroke (35), have been shown to disrupt BBB structural integrity, leading to functional breakdown.

Many studies measure BBB disruption by increased permeability of the microvasculature to albumin (31, 59). We argue that by the time albumin, a 65,000-Da protein, is measurable in the brain parenchyma, the BBB is already compromised. Rather, we contend that changes in BBB function using much smaller vascular space markers, such as sucrose (342 Da) and insulin (5,000 Da), provide an intriguing opportunity to investigate the regulatory properties of the tight junction and adjacent extracellular matrix during a pathological insult and may identify future therapeutic targets. Morphologically, BBB microvasculature has shown signs of diabetes-induced angiopathy, with increased vesicle formation and serum albumin staining in the Virchow-Robin space (8). Recent studies demonstrated that small openings in the BBB can have a significant impact on BBB function and structure. Using magnetic resonance imaging on patients with Type II diabetes, investigators showed increased BBB permeability to gadolinium-diethylene-triamine pentaacetic acid (DTPA) and concluded that, although the openings in the BBB were to a small molecule (gadolinium-DTPA; 570 Da), clinical significance was substantial because these effects may play a role in the increased progressive
cognitive impairment often seen in patients with diabetes (56). Additionally, a recent study showed that streptozotocin (STZ)-induced diabetes in rats altered the molecular structure of BBB tight junctions by decreasing the expression of occludin, with no change in the accessory protein zonula occludens 1 (11).

Because of the progressive nature of diabetes and the unique phenotype of the BBB, the effects of diabetes on the cerebrovascular microvasculature are different from other microvascular beds and barrier systems, such as seen at the retina and peripheral nerves. Adverse effects at the BBB may be more insidious because vascular dysregulation is less perceptible at first, and by the time clinical signs are noticeable, irreversible neurological damage may have occurred. We hypothesize that diabetes has a long-term, progressive effect on BBB endothelial cells, resulting, at first, in small, transient breaches that, over time, grow larger and more pronounced.

RESEARCH DESIGN AND METHODS

Chemicals and radioisotopes. STZ, regular insulin, Evans blue, and reagent-grade chemicals were purchased from Sigma Chemical (St. Louis, MO). [14C]sucrose (specific activity: 485 mCi/mmol, >99.5% purity) and [3H]inulin (specific activity: 355 mCi/g, >99% purity) were purchased from MP Biomedical (Costa Mesa, CA). [3H]butanol (specific activity: 20 Ci/mmol, >99% purity) was purchased from American Radiolabeled Chemicals (St. Louis, MO).

Animals. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 250–274 g were housed under 12-h:12-h light-dark conditions and received food and water ad libitum. Animals were acclimatized to the environment for 7 days before induction of diabetes. All protocols involving animals were approved by the West Virginia University Animal Care and Use Committee and abide by National Institutes of Health guidelines.

Diabetic induction procedures. STZ was dissolved in sodium citrate (50 mM; pH 4.5)-buffered 0.9% saline, and regular insulin was dissolved in 0.9% saline. Rats were divided into three treatment groups. Group I received a single injection of sodium citrate-buffered 0.9% saline and served as the control. Group II received a single injection (intraperitoneally) of STZ (60 mg/kg; 100 μl). Group III received a single injection of STZ (60 mg/kg; 100 μl) and then received regular insulin (4 U/kg sc) twice daily upon determination of hyperglycemia. Glucose water (10%) was put into cages of rats given STZ for 12 h to protect against STZ-induced hypoglycemia. Animals were classified as diabetic if blood glucose level measured >350 mg/dl, and only animals with a blood glucose level >350 mg/dl were allowed to continue in groups II and III.

Experimental procedures. Animal studies were conducted at 7, 28, 56, and 90 days. Blood glucose and weight were measured before the start of the studies. Animals from each group were assessed for BBB permeability to Evans blue albumin (65,000 Da), [3H]inulin (5,000 Da), and [14C]sucrose (342 Da). To determine localization of changes in BBB permeability, rat brains were dissected on ice (in the following order): hypothalamus, cerebellum, midbrain, cerebral cortex, hippocampus, basal ganglia, and thalamus.

Evans blue extravasation. For quantification of albumin extravasation, rats were anesthetized with pentobarbital sodium (60 mg/kg ip), and 2% Evans blue (4 ml/kg; 1 ml) was infused via the femoral artery and allowed to circulate for 1 h. Rats were perfused with cold phosphate-buffered saline with heparin (2 U/ml; pH 7.4) for 15 min via the left ventricle. After perfusion, rats were killed by decapitation and the brain was extracted. Excised brain was weighed, dissected, and homogenized in 500 μl of 50% trichloroacetic acid. Tissue was incubated for 24 h at 37°C. At 24 h, samples were centrifuged at 13,000 g for 10 min and the supernatants were diluted fourfold with absolute ethanol. Fluorescence intensity was measured using a spectrofluorometer at 620-nm excitation, 680-nm emission (RF 5301 PC; Shimadzu, Columbia, MD). Calculations were based on external standard readings, and extravasated dye was expressed as nanograms of Evans blue per milligram brain tissue.

In situ brain perfusion. In situ brain perfusion studies were carried out based on the method of Preston et al. (46). Briefly, rats were anesthetized with intramuscular injection of rat cocktail (2.5 mg/kg flumoxine, 90 mg/kg ketamine, and 5 mg/kg xylazine) and heparinized (10,000 U/kg ip), and body temperature was maintained at 37°C. Common carotid arteries were exposed, and right common carotid was cannulated and perfused with an erythrocyte-free perfusion media consisting of a modified Krebs-Henseleit Ringer solution [117 mM NaCl; 4.7 mM KCl; 0.8 mM MgSO4, 24.8 mM NaHCO3, 1.2 mM KH2PO4, 2.5 mM CaCl2, 10 mM d-glucose, 29 g/l dextran (70,000 Da), and 1 g/l bovine serum albumin], which was aerated with 95% O2-5% CO2 and warmed to 37°C. With the start of the perfusion, the right jugular vein was sectioned to allow for drainage. Once the desired perfusion pressure (85–95 mmHg) and flow rate (3.1 ml/min) were achieved for right common carotid artery, the contralateral carotid artery was cannulated and perfused in a similar manner. Once both arteries were cannulated, radiolabeled compound was infused via a slow-drive syringe pump (flow rate: 0.5 ml/min; model 22, Harvard Apparatus) into the inflowing mammalian Ringer solution (total flow rate: 3.6 ml/min per hemisphere). After 20 min, brain was flushed for 20 s with unlabeled Ringer solution and the animal was decapitated. The brain was removed, and choroid plexuses and meninges were excised. The brain was dissected into brain regions as described in Experimental procedures and homogenized. Perfusion fluid was collected from carotid cannula by briefly resuming perfusion of radiolabeled compound following termination. Brain tissue samples (~500 mg wet wt) and 100 μl of perfusate samples were prepared for radioactive counting by addition of 1 ml of tissue solubilizer (TS-2; Research Products, Mount Prospect, IL); 30 μl of glacial acetic acid (to quench chemiluminescence) and 4 ml of scintillation cocktail (Budget Solve; Research Products) were then added, and samples were analyzed by liquid scintillation counting on a Beckman LS5801 (Beckman Coulter, Fullerton, CA). Amount of [3H] and [14C] radioactive activity in the brain ([Ctissue, in disintegrations per minute (dpm)] per gram (dpm/g)) was expressed as a percentage of that in the artificial perfusate ([Cperfusate, in dpm/ml]) and termed Rtissue% (in μg/l) as follows: Rtissue% = ([Ctissue] × [Cperfusate]) / 100,000.

Capillary depletion studies. Capillary depletion method was based on the method of Triguero et al. (60). After in situ perfusion, brain was removed and choroid plexuses and meninges were excised, dissected as described in In situ brain perfusion, and homogenized in 1.5 ml of capillary depletion buffer (4-2-hydroxyethyl)-1-piperazineethane sulfonic acid; 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl2, 1 mM MgSO4, 1 mM NaH2PO4, and 10 mM d-glucose; pH 7.4) and kept on ice. Two milliliters of ice-cold dextran (60,000 Da) solution were added to homogenate. Two aliquots of homogenate were taken and centrifuged at 5,400 g for 15 min. Capillary-depleted supernatant was separated from vascular pellet. Homogenate, supernatant, and pellet were counted for radioactivity on scintillation counter. All homogenization procedures were carried out within a 2-min time span.

Measurement of cerebral blood flow. The perfusion method of Preston et al. (46) was adapted to determine both cerebral blood flow (CBF) and rate of cerebral perfusion in situ to determine the [3H]butanol uptake, using derived equations of Gjedde and Crone (18). In situ brain perfusion was carried out as stated above with a Ringer solution containing 4 ml/l unlabeled ethanol. With the use of a slow-drive syringe pump (0.5 ml/min per hemisphere), [3H]butanol was added during last 10 s of a 20-min perfusion. A partition coefficient (λbr) was determined by using a separate group of animals (n = 3) for each treatment and time that was perfused with a constant [3H]butanol concentration in arterial inflow for 20 min followed by brain sampling and analysis. After perfusion, brains were weighed and sectioned. Brain and Ringer solution samples were taken for liquid scintillation counting. A small portion of frontal lobes (~50 mg) was
removed and weighed separately to determine the brain tissue dry weight by drying in an oven at 95°C to constant weight. Unlabeled ethanol was added to saturate endogenous alcohol dehydrogenase for both measurements.

Calculation of CBF. Measurement of CBF was quantified by using the derived equation from Gjedde and Crone (18): 

$$F_{bl} = -\lambda_{br} \ln[(1 - C_{br}/C_{br}^{0})/t]$$

where $F_{bl}$ is rate of blood flow [in ml/min/unit mass$^{-1}$ (g)] and $C_{br}$ is the constant $[^{3}H]$butanol concentration in arterial inflow at time $t$, $\lambda_{br}$ is the constant $[^{3}H]$butanol concentration in arterial inflow at time $t$ between introduction of $[^{3}H]$butanol and decapitation. $C_{br}$ is activity in unit weight of brain at time $t$; $\lambda_{br}$ is the derived equation from Gjedde and Crone (18): 

$$F_{bl} = C_{br} \lambda_{br}$$

results were analyzed using one-way ANOVA with Tukey’s post hoc test.

RESULTS

Determination of Evans blue extravasation. BBB permeability to albumin (65,000 Da) was measured in whole brain of age-matched vehicle-treated (group I), diabetic (group II), and insulin-treated diabetic (group III) rats at 7, 28, 56, and 90 days by quantification of Evans blue. Evans blue binds with affinity to albumin and is a commonly used tool to quantify albumin extravasation in tissue and predict edema formation. Results showed no significant ($P > 0.05$) difference in total Evans blue extravasation in whole brain between any treatment group at any time point (Fig. 1A). No significant ($P > 0.05$) interaction between treatment and day was observed.

Determination of regional differences in Evans blue extravasation. Regional differences in BBB permeability to albumin (65,000 Da) were measured in rats from groups I, II, and III at 7, 28, 56, and 90 days by quantification of Evans blue (Table 1). Results showed that extravasation of Evans blue was not significantly ($P > 0.05$) different between groups I, II, and III at 7, 28, and 56 days. At 90 days, groups II and III exhibited a significant ($P < 0.05$) increase in Evans blue extravasation in the midbrain ($1.0 \pm 0.2$ and $0.9 \pm 0.1$, respectively) as compared with group I ($0.3 \pm 0.1$). Moreover, group II had a significant ($P < 0.05$) increase in Evans blue extravasation in the basal ganglia ($1.5 \pm 0.2$) as compared with group I ($1.0 \pm 0.1$) at 90 days. No significant ($P > 0.05$) interaction between treatment and day within a region was observed.

In situ brain perfusion using $[^{3}H]$inulin. BBB permeability was assessed in whole brain of rats from groups I, II, and III at 7, 28, 56, and 90 days after STZ induction by in situ brain perfusion with an impermeant marker ($[^{3}H]$inulin; 5,000 Da) over 20 min. Results showed no significant difference ($P > 0.05$) in inulin associated with total brain between the treatment groups (groups II and III) as compared with group I at any time point (Fig. 1B). No significant ($P > 0.05$) interaction between treatment and day was observed.

Determination of regional differences in $[^{3}H]$inulin association with brain. Using in situ brain perfusion with $[^{3}H]$inulin, we assessed rats in groups I, II, and III for changes in permeability of inulin into different brain regions at 7, 28, 56, and 90 days following STZ-induced diabetes (Table 2). Results showed no significant ($P > 0.05$) change in overall BBB permeability to $[^{3}H]$inulin; however, a few brain regions of groups II and III exhibited a significant ($P < 0.05$) increase in inulin associated with the brain parenchyma as compared with those regions in group I. A significant ($P < 0.05$) increase in inulin permeability across the BBB was observed in the cerebral cortex of group II at 56 days ($2.5 \pm 0.2$) and 90 days ($2.5 \pm 0.2$) as compared with group I ($1.8 \pm 0.1$ and $1.8 \pm 0.1$, respectively). Group III showed increased permeability of inulin across the BBB in the cerebral cortex at 90 days ($2.4 \pm 0.1$) as compared with group I ($1.8 \pm 0.1$). Groups II and III exhibited increased inulin permeability across the BBB in the midbrain at 56 days ($2.1 \pm 0.3$ and $1.8 \pm 0.1$, respectively) and 90 days ($2.3 \pm 0.1$ and $2.0 \pm 0.1$, respectively) as compared with group I ($1.1 \pm 0.1$ and $1.1 \pm 0.1$, respectively). A
permeability of sucrose into different brain regions at 7, 28, 56, and 90 days following STZ-induced diabetes (Table 3). Results showed a significant ($P < 0.05$) increase in BBB permeability to $[14C]$sucrose in a number of brain regions of groups II and III when compared with group I. A significant ($P < 0.05$) increase in sucrose permeability was observed in the cerebral cortex of group II at 28 days ($2.5 \pm 0.3$) and 56 days ($4.5 \pm 0.3$) as compared with both groups I ($2.0 \pm 0.2$ and $2.0 \pm 0.2$, respectively) and III ($2.0 \pm 0.2$ and $2.4 \pm 0.2$, respectively). No significant ($P > 0.05$) difference was observed between the cortex of groups I and III at 7, 28, and 56 days. At 90 days, groups II and III demonstrated a significant ($P < 0.05$) increase in sucrose permeability in the cerebral cortex as compared with group I. No significant ($P > 0.05$) difference between groups II and III was observed in the cortex at 90 days. Group II exhibited a significant ($P < 0.05$) increase in sucrose associated with the brain parenchyma in the hippocampus at 56 days ($2.4 \pm 0.1$) and 90 days ($2.8 \pm 0.2$) as compared with groups I ($1.5 \pm 0.1$ and $1.5 \pm 0.1$, respectively) and III ($1.7 \pm 0.2$ and $1.8 \pm 0.2$, respectively). At 28, 56, and 90 days, groups II ($3.1 \pm 0.3$, $3.5 \pm 0.4$, and $4.6 \pm 0.5$, respectively) and III ($2.1 \pm 0.3$, $2.8 \pm 0.3$, and $3.7 \pm 0.5$, respectively) showed a significant ($P < 0.05$) increase in sucrose permeabil-
The main findings of this study were that STZ-induced diabetes produced a progressive increase in BBB permeability to small molecules from 28 to 90 days and that these changes in BBB permeability were region specific, with the midbrain seemingly most susceptible to diabetes-induced microvascular damage. Furthermore, this study showed that increased association of a vascular space marker with the brain parenchyma pressures and rates showed no difference between groups II and III as compared with control (group I) at 7, 28, 56, and 90 days. CBF (Fb0) was calculated at t = 10 s in situ in brain perfusion with [3H]butanol. Results showed no significant (P > 0.05) change in CBF in the treatment groups (groups II and III) as compared with control (group I). Brain weights and the percent water content were similar among all treatment groups (groups II and III) compared with control (group I).
Table 5. Cerebral blood flow analyses using in situ brain perfusion with \[^{3}H\]butanol and measurement of percent brain water in rats at 7, 28, 56, and 90 days following STZ-induced diabetes

<table>
<thead>
<tr>
<th>Day</th>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>(\lambda_{be})</td>
<td>0.72</td>
<td>0.75</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Perfusion pressure, mmHg</td>
<td>83.4 ± 4.8</td>
<td>82.8 ± 3.1</td>
<td>83.8 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Perfusion rate, ml/min (\text{g}^{-1})</td>
<td>1.70 ± 0.03</td>
<td>1.69 ± 0.07</td>
<td>1.73 ± 0.02</td>
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<tr>
<td></td>
<td>Cerebral blood flow, ml/min (\text{g}^{-1})</td>
<td>1.22 ± 0.31</td>
<td>1.35 ± 0.21</td>
<td>1.28 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>Perfused brain weight, g</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Perfused brain water, %</td>
<td>82.8 ± 0.4</td>
<td>83.1 ± 0.7</td>
<td>82.7 ± 0.7</td>
</tr>
<tr>
<td>28</td>
<td>(\lambda_{be})</td>
<td>0.69</td>
<td>0.74</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Perfusion pressure, mmHg</td>
<td>81.5 ± 3.6</td>
<td>84.2 ± 4.7</td>
<td>83.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Perfusion rate, ml/min (\text{g}^{-1})</td>
<td>1.66 ± 0.04</td>
<td>1.74 ± 0.06</td>
<td>1.71 ± 0.02</td>
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<tr>
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<td>Cerebral blood flow, ml/min (\text{g}^{-1})</td>
<td>1.15 ± 0.24</td>
<td>1.26 ± 0.34</td>
<td>1.20 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Perfused brain weight, g</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Perfused brain water, %</td>
<td>82.1 ± 1.2</td>
<td>82.7 ± 0.8</td>
<td>83.2 ± 0.9</td>
</tr>
<tr>
<td>56</td>
<td>(\lambda_{be})</td>
<td>0.71</td>
<td>0.80</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Perfusion pressure, mmHg</td>
<td>83.4 ± 2.8</td>
<td>81.7 ± 3.7</td>
<td>86.2 ± 3.3</td>
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<tr>
<td></td>
<td>Perfusion rate, ml/min (\text{g}^{-1})</td>
<td>1.71 ± 0.05</td>
<td>1.76 ± 0.04</td>
<td>1.68 ± 0.05</td>
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<td>Cerebral blood flow, ml/min (\text{g}^{-1})</td>
<td>1.31 ± 0.25</td>
<td>1.27 ± 0.19</td>
<td>1.22 ± 0.29</td>
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<tr>
<td></td>
<td>Perfused brain weight, g</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Perfused brain water, %</td>
<td>81.4 ± 0.8</td>
<td>81.2 ± 1.1</td>
<td>82.4 ± 0.7</td>
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<tr>
<td>90</td>
<td>(\lambda_{be})</td>
<td>0.73</td>
<td>0.74</td>
<td>0.73</td>
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<tr>
<td></td>
<td>Perfusion pressure, mmHg</td>
<td>87.1 ± 3.6</td>
<td>83.4 ± 4.7</td>
<td>85.5 ± 3.9</td>
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<td>Perfusion rate, ml/min (\text{g}^{-1})</td>
<td>1.72 ± 0.04</td>
<td>1.78 ± 0.09</td>
<td>1.67 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Cerebral blood flow, ml/min (\text{g}^{-1})</td>
<td>1.14 ± 0.26</td>
<td>1.34 ± 0.19</td>
<td>1.26 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Perfused brain weight, g</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Perfused brain water, %</td>
<td>81.0 ± 0.4</td>
<td>81.3 ± 0.6</td>
<td>81.7 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 6 rats. Group I, control; Group II, diabetes; Group III, diabetes + insulin; \(\lambda_{be}\), partition coefficient.

was due to transfer from the systemic circulation to the brain and not increased trapping or endocytosis into brain capillary endothelial cells. In addition, this study showed that insulin treatment of diabetes attenuated BBB disruption, especially during the first few weeks; however, as diabetes progressed, it was evident that microvascular damage occurred even when hyperglycemia was controlled. Finally, this study demonstrated that STZ-induced diabetes did not alter total CBF or edema formation at any time point evaluated; therefore, it is improbable that these factors played a role in the increased association of radiolabeled vascular space marker with the brain.

STZ induced hyperglycemia in >95% of rats by 24 h after administration. Availability of 10% glucose water was used to alleviate potential severe hypoglycemia in the rats as a result of increased insulin release during the destruction of pancreatic \(\beta\)-cells. To limit episodes of hypoglycemia in group III animals, insulin treatment was divided into two doses and glucose levels were regularly monitored. With the use of this approach, group III animals had a fasting blood glucose level of 128 ± 19 mg/dl as compared with 146 ± 10 mg/dl in group I and 426 ± 31 mg/dl in group II animals.

Changes in BBB permeability due to STZ-induced diabetes were assessed by using three varying sized vascular space markers [albumin (65,000 Da), inulin (5,000 Da), and sucrose (342 Da)]. Moreover, using differential permeability of radiolabeled vascular space markers has been effective at measuring regional changes in brain uptake (40, 41, 66). Assessment of albumin extravasation is a frequently used method to measure vascular leakage. The intact BBB has negligible transport of albumin into the brain and serves as a physical barrier to partition the systemic circulation from the brain parenchyma. However, susceptibility of the BBB to increased permeability has been documented in a number of diseases. Whereas diabetic-induced microvascular leakage is commonly associated with early endothelial dysfunction of the retina (14), kidney (10), and peritoneum (64), few studies have documented changes in the brain. One possible reason for this may be due to BBB phenotype and the relative resistance of the BBB to damage as compared with other microvascular areas. In this study, we measured albumin extravasation into the brain parenchyma by quantifying the amount of Evans blue albumin extracted from the brains of rats in groups I, II, and III. No change in total albumin extravasation was observed in the treatment groups (II and III) as compared with control rats, thus suggesting that the BBB remained intact. This observation was reinforced by measurements showing no change in the percentage of brain water, a hallmark indicator of increased extracellular albumin and edema formation. Whereas total albumin extravasation remained unchanged, on closer evaluation of brain regions, we noted a significant increase in Evans blue albumin in the midbrain of groups II and III at 90 days and in the basal ganglia of group II at 90 days. These results suggest that the BBB is not a homogenous membrane but, rather, may have areas of increased susceptibility. Moreover, these results argue in favor of our rationale that, by the time albumin is measured, the BBB is compromised and therefore assessment of smaller vascular markers would be more indicative of how the BBB is responding to diabetes-related vascular complications.

In situ brain perfusion, which was used to measure changes in BBB permeability to inulin and sucrose, is a methodology that has successfully assessed drug transport across the BBB into the CNS (15, 22, 33) and, more recently, changes in BBB permeability due to diabetes.
function following pathology (16, 23, 26, 63). In situ brain perfusion has been shown to maintain good neurological function and other physiological factors in rat and guinea pig models (46, 68), and, when coupled to capillary depletion, these techniques are precise tools for assessing BBB function. Using these techniques, we determined that BBB functional integrity was maintained to molecules >5,000 Da out to 90 days of STZ-induced diabetes; however, BBB functional integrity was disrupted to small molecules by 28 days and progressively worsened out to 90 days. In addition, our studies indicated that control of STZ-induced diabetes with insulin treatment attenuated BBB disruption to sucrose. However, as diabetes progressed, insulin treatment in group III did not effectively reduce the increased BBB permeability to sucrose observed at 56 and 90 days. The finding that BBB disruptions increased over time supports our rationale that effects of diabetes on the cerebral microvasculature occur in a more insidious manner than effects of other microvascular deficits. Furthermore, since diabetes is a lifelong disease, occurring more prevalently in younger populations, the long-term effects of diabetes on brain function are of paramount importance.

We assessed whether diabetes-induced BBB permeability to inulin and sucrose was occurring globally or in specific brain regions. Early changes in microvascular permeability noted in other tissues were not apparent in the BBB, even to small molecules. Although no change in inulin permeability was observed in the total brain at any time point assessed, when brain regions were evaluated separately, we observed increased permeability to inulin in the cerebral cortex and midbrain in group II at 56 and 90 days, in the basal ganglia of group II at 90 days, and in the midbrain of group III at 90 days. Assessment of brain region-specific permeability of sucrose demonstrated a greater distribution of sucrose than observed with inulin. We observed increased permeability in the cortex at 28, 56, and 90 days in group II and increased permeability in the midbrain, hippocampus, and basal ganglia in group II at 56 and 90 days. Group III showed an increased permeability in the midbrain at 56 and 90 days and in the cerebral cortex and basal ganglia at 90 days. These findings suggest that the BBB has certain areas more susceptible to breakdown than others. On the basis of our findings and the current literature, we hypothesize that BBB disruptions observed in this study were due to changes in cell-cell contacts leading to increased paracellular flux; however, the possibility exists that these changes were due to alterations in transcytotic pathways or increased capillary fenestrations, and future studies will need to address this important issue.

Findings that BBB permeability changes were regional rather than global are not surprising, and yet this area has been understudied with regard to the effects of diabetes. CBF and capillary density are not evenly distributed in white and gray matter areas of the brain (4). Under basal conditions, areas with higher metabolic need (i.e., greater demand for glucose) have greater capillary density and increased CBF. However, during CNS pathology, the area of the brain affected has a large influence on the ability for recovery. Results from this study suggest a differential susceptibility to diabetes-induced BBB disruption in brain regions. BBB disruptions in the midbrain, an area with lower capillary density and CBF than the cortex, were observed at 28 days and were larger in size than seen in other brain areas. Clinical case reports cite an increased susceptibility to third nerve palsies in diabetics due to an increased prevalence of midbrain lesions and hemorrhaging (24, 37). Furthermore, diabetes-induced lesions have been reported to attenuate morphine analgesia due to decreased serotoninergic activity in the raphe magnus nucleus (48, 55). Of particular interest in this investigation was the finding that other brain areas with greater cerebral flow were affected as diabetes progressed to 56 and 90 days. These findings suggest that diabetes-related changes to the CNS (i.e., increased oxidative stress, decreased vascular reactivity, and altered access to metabolic substrates) have a cumulative effect that takes a greater period of time to manifest in altered BBB function.

The “neurovascular unit,” which is composed of cerebral endothelial cells, pericytes, glia, and neurons, carefully orchestrates localized changes in CBF to rapidly meet metabolic demands. Under basal physiological conditions, the spatial and temporal relationship between neural activity and CBF, termed neurovascular coupling, utilizes cerebrovascular changes induced by activation to represent changes in function in the human brain (19, 32). Moreover, CBF is maintained in a narrow range by autoregulatory mechanisms, irrespective of changes in systemic blood flow (50). However, in several brain pathologies, interactions between neural activity and cerebral blood vessels are disrupted, and the resulting homeostatic imbalance, known as neurovascular uncoupling, may contribute to brain dysfunction, including but not limited to BBB disruptions. In Alzheimer’s disease, hypertension, and ischemic stroke, cerebrovascular function is altered, resulting in reduced CBF, altered autoregulation, disruption in nutrient trafficking across the BBB, and attenuated response to increased metabolic demand (7, 19, 38, 42). Often, these changes in cerebrovascular function precede onset of any cognitive impairment, suggesting a role for neurovascular uncoupling in the etiology and progression of cognitive dysfunction. Using these concepts, we argue that observed regional changes in BBB function were due to a number of factors (differential neuronal viability, increased metabolic demand, and oxidative stress) in specific brain regions brought about by diabetes-associated effects, including hyperglycemia, dyslipidemia, and increased cholesterol (15, 17). Future studies would be well served to gain a better understanding of how neural and glia cells respond to diabetes and how changes in these cells affect cerebrovascular function.

CBF in control rats (group I) using [3H]butanol was consistent with previously reported values ranging from 0.8–1.49 ml·min⁻¹·g⁻¹ (Table 5) (28). The current study showed no change in CBF at any time point assessed regardless of treatment. A number of factors associated with diabetes have been found to play an integral role in cerebrovascular changes, including oxidative stress, hyperglycemia, atherosclerosis, and autonomic dysfunction (5, 21, 45, 47). Results of this study suggest cerebral autoregulation of blood flow in the whole brain remained intact during diabetes, which further reafirms that the increased association of the vascular space marker with the brain parenchyma was due to changes in BBB functional integrity and not changes in hemodynamics. However, what cannot be extrapolated from our findings is possible regional breakdowns in cerebral autoregulation (i.e., neurovascular uncoupling) and the cerebral vascular response to further stressors, such as an acute hypertensive state or additional oxidative stress in the STZ-treated groups (groups I and II). Future
studies are needed to evaluate the ability of cerebral microvessels to adapt and respond to stressors in diabetic rats.

In summary, we have shown that, as diabetes progresses, the extent of microvascular leakage to small molecules increases. These results suggest that diabetes-induced perturbations to cerebral microvessels may disrupt homeostasis and contribute to long-term cognitive and functional deficits. The BBB perturbations observed are to small molecules; nonetheless, these disruptions are significant, as noted in the gadolinium-DTPA study (56), due to the correlation between increased BBB permeability, altered CNS homeostasis, and impaired neuronal function. Interestingly, changes in BBB permeability were region specific, and many of the areas affected can be associated with detrimental CNS outcomes, including the midbrain, basal ganglia, cortex, and hippocampus. Thus these results put forward the rationale that microangiopathy of the cerebrovasculature may play a primary role in the etiology of diabetes-induced CNS disorders. Further studies will be needed to evaluate the role of BBB endothelial cell tight junction regulation and basement membrane alterations in increased microvascular permeability and focus on how alterations in neurovascular unit function in the identified brain regions relate to the etiology of adverse CNS effects.

GRANTS

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REFERENCES


