Experimental colitis increases blood-brain barrier permeability in rabbits

CHRISTOPHER A. HATHAWAY, CAROLINE B. APPLEYARD, WILLIAM H. PERCY, AND JOHN L. WILLIAMS

Department of Physiology and Pharmacology, School of Medicine, The University of South Dakota, Vermillion, South Dakota 57069

Hathaway, Christopher A., Caroline B. Appleyard, William H. Percy, and John L. Williams. Experimental colitis increases blood-brain barrier permeability in rabbits. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1174–G1180, 1999.—Extraintestinal manifestations of inflammatory bowel disease are numerous. This study examined the effects of two models of acute colitis on cerebral blood flow (CBF) and permeability of the blood-brain barrier in rabbits. CBF (measured with radiolabeled microspheres), or the extraction ratio or permeability-surface area (PS) product of the blood-brain barrier to fluorescein and FITC-dextran, was measured 48 h after colitis induction with acetic acid (HAc) or trinitrobenzene sulfonic acid (TNBS). PS product for fluorescein increased (P < 0.05) in TNBS colitis (1.33 × 10⁻⁵ ± 0.52 × 10⁻⁵ mls and 0.48 × 10⁻⁵ ± 0.13 × 10⁻⁵ mls; mean ± SE) for treated (n = 14) and untreated (n = 10) animals, respectively. PS product for the larger FITC-dextran was not different in TNBS colitis (0.24 × 10⁻⁵ ± 0.09 × 10⁻⁵ mls, n = 7) compared with untreated controls (0.19 × 10⁻⁵ ± 0.04 × 10⁻⁵ mls, n = 8). PS product for fluorescein increased (P < 0.01) in HAc colitis compared with vehicle (2.66 × 10⁻⁵ ± 1.46 × 10⁻⁵ mls and 0.33 × 10⁻⁵ ± 0.05 × 10⁻⁵ mls, respectively; n = 6 in each group). The extraction of fluorescein from the blood to the brain increased by 75% during TNBS colitis when compared with vehicle (P < 0.05). CBF and cerebrovascular resistance did not change from the untreated control after TNBS colitis. Our data suggest that, irrespective of induction method, acute colitis increases the permeability of the blood-brain barrier to small molecules without changing CBF.

Cerebral blood flow; brain-gut axis; colitis model; inflammatory bowel disease; vasculature

MAINTENANCE OF AN INTACT blood-brain barrier is essential for proper function of the central nervous system. The blood-brain barrier is present in almost all regions of the brain and is composed primarily of endothelial cells. The endothelial cells that line the blood vessels of the brain form tight junctions and normally have very low pinocytotic activity when in the presence of the supporting astrocytes, pericytes, and microglia (4, 34, 39). The blood-brain barrier, therefore, physically inhibits many large or hydrophilic molecules in the blood from entering the brain parenchyma (4, 34). Some molecules that are essential for proper brain function circumvent the blood-brain barrier via highly regulated specific transport proteins (4). Thus the blood-brain barrier is a major contributor to homeostasis of the brain milieu.

Inflammatory responses are associated with many hematologic changes that can affect blood flow and vascular permeability at the site of inflammation. For example, meningitis causes an increase in the permeability of the blood-brain barrier, and changes in gastrointestinal vascular permeability are common in patients with inflammatory bowel disease (IBD) (26, 29). Cardiovascular responses to IBD, however, may not be limited to the site of inflammation in this disease. Many extracolonic manifestations of IBD are vascular in nature, such as eosinophilic pneumonia and bronchiectasis, which are both associated with increased permeability of the pulmonary vasculature during colitis (14, 16, 27). Colitis also increases the permeability of the renal vasculature, as demonstrated by the high correlation between microalbuminuria and disease activity and tumor necrosis factor-α (TNF-α) levels in the blood (22, 23).

Increases in the number of circulating platelets and decreases in the number of circulating red blood cells are generally correlated with the activity and extent of IBD (19, 31). Increases in blood clotting activity during colitis can cause deep vein thrombosis, which in turn can lead to pulmonary embolism or stroke (7, 18, 31, 35, 36). Other vascular abnormalities associated with IBD include cutaneous vasculitis, arteritis, and signs of peripheral and cerebrovascular endothelial lesions (13, 35, 36). In addition, factors involved in blood clot formation can have effects on vascular permeability. For example, thrombin increases permeability of the blood-brain barrier in vitro, possibly through the release of nitric oxide (9).

The purpose of this study was to evaluate the effects of acute colitis on blood-brain barrier permeability and to determine whether these changes were related to alterations in cerebral blood flow. We hypothesized that active inflammation of the colon would increase the permeability of the blood-brain barrier to molecules that would normally be restricted. In our studies, blood-brain barrier permeability was examined in two different models of colitis in rabbits. Extravasation of tracer from plasma to cerebrospinal fluid was measured as an index of permeability. Cerebral blood flow was measured with radiolabeled microspheres.

MATERIALS AND METHODS

Preparation of animals. The experiments reported herein were performed in accordance with the principles described in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH)
Male New Zealand White rabbits (~2.5 kg) were tranquilized with ketamine and xylazine (5 mg/kg im of each). Animals were randomly assigned to treatment groups. Colonic inflammation was induced using modified versions of the methods of Morris et al. (28) and Fretland et al. (12). Briefly, a catheter was advanced rectally into the colon until its tip was ~8 cm above the anus. Acetic acid (HAc; 1 ml of 5% acetic acid-30% ethanol), trinitrobenzene sulfonic acid (TNBS; 150 mg dissolved in 1 ml of 50% ethanol), or vehicle (1 ml of a 30% or 50% ethanol solution) was instilled into the lumen of the colon through the catheter. The animals were then allowed to recover and had free access to food and water. Anti-inflammatory drugs and other analgesics were not used in this study because they could interfere with the pathophysiological mechanisms that were being investigated. The animals were closely monitored and euthanized if they showed signs of undue distress from the treatment.

Forty-eight hours after colonic treatment, rabbits were anesthetized with a mixture of xylazine and ketamine (30 mg/kg im of each). Supplemental anesthesia (pentobarbital sodium, 5 mg/kg iv) was given when pressure on a paw caused paw withdrawal or changes in arterial blood pressure or heart rate. The right femoral vein and artery were cannulated for administration of drugs and measurement of blood pressure, respectively. The thoracic aorta was cannulated via the left femoral artery for withdrawal of reference blood samples. The animals were paralysed with gallamine triethiodide (5 mg/kg iv) after they were artificially ventilated with air that was supplemented with oxygen. Arterial blood gases and pH were kept within physiologically normal levels (P'O₂ = 100–150 mmHg, P'CO₂ = 33–37 mmHg, pH = 7.35–7.45).

Forty-eight hours after colitis induction was selected as the optimum time for these experiments because fully developed inflammation was first seen consistently at this time in preliminary experiments. After each experiment, the anesthetized rabbit was euthanized by an intravenous overdose of potassium chloride, and the colon was removed and macroscopically graded (37) for damage by a blinded observer (Table 1) as described by Bell et al. (3).

Table 1. Criteria for macroscopic scoring of colonic damage

<table>
<thead>
<tr>
<th>Score</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Localized hyperemia, no ulcers</td>
</tr>
<tr>
<td>2</td>
<td>Ulceration without hyperemia or bowel wall thickening</td>
</tr>
<tr>
<td>3</td>
<td>Ulceration with inflammation at one site</td>
</tr>
<tr>
<td>4</td>
<td>Two or more sites of ulceration and inflammation</td>
</tr>
<tr>
<td>5</td>
<td>Major sites of damage extending &gt; 1 cm along length of colon</td>
</tr>
<tr>
<td>6-10</td>
<td>Major sites of damage extending &gt; 2 cm along length of colon, with score increasing by 1 for each additional cm</td>
</tr>
<tr>
<td></td>
<td>Other considerations</td>
</tr>
<tr>
<td></td>
<td>Thickness of the colon (in mm)</td>
</tr>
<tr>
<td></td>
<td>+1 If minor adhesions are present (colon can be easily separated from other tissues)</td>
</tr>
<tr>
<td></td>
<td>+2 If major adhesions are present</td>
</tr>
<tr>
<td></td>
<td>+1 If animal has diarrhea</td>
</tr>
</tbody>
</table>

Total

Measurement of blood-brain barrier permeability. In four series of experiments, permeability of the blood-brain barrier was examined. In the first three series, following the general preparation described in Preparation of animals, a cranial window was formed by drilling a circle in the skull (~2 cm diameter) with an air-cooled drill, and the bone and dura mater were removed. Dental acrylic was used to form a dam around the craniostomy, and warm (37°C) artificial cerebrospinal fluid (CSF) that was bubbled with 95% N₂-5% CO₂ was superfused over the brain at 1–2 ml/min. After 30 min of recovery, control samples of arterial blood and CSF were taken, and the experimental protocol was performed. The experiment was not performed if any bleeding was present in the cranial window.

In the first series of experiments, 5% sodium fluorescein (mol wt 376) was given intravenously (2.5 ml/kg) in rabbits treated with either HAc (n = 6) or 30% ethanol vehicle (n = 6). After the injection of fluorescent tracer, arterial blood and artificial CSF samples were taken at 5-min intervals for 35 min. The goal of these experiments was to determine whether HAc-induced colitis increases the permeability of the blood-brain barrier to fluorescein, a relatively small molecule.

In the second series of experiments, blood-brain barrier permeability to 5% sodium fluorescein in rabbits treated with TNBS (n = 14) or in untreated rabbits (n = 10) was measured. In these experiments, arterial blood and artificial CSF samples were taken 20 min after a 5% sodium fluorescein injection (2.5 ml/kg iv). These experiments were performed to confirm that the altered blood-brain barrier permeability was a feature of acute colitis itself and not an artifact of the method of colitis induction.

In the third series of experiments, 5% FITC-dextran (mol wt 71,200; 2.5 ml/kg iv) was used to measure the permeability of the blood-brain barrier to large molecules in rabbits treated with TNBS to induce colitis (n = 8) and in untreated rabbits (n = 7). Permeability to FITC-dextran was measured 20 min after the FITC-dextran was injected intravenously (calculated from a blood and artificial CSF sample). These experiments were performed to determine whether TNBS causes increases in permeability of the blood-brain barrier to large molecules.

At the end of these experiments, blood samples were centrifuged, and plasma was extracted and diluted by a factor of 10,000. The CSF and diluted blood samples were then measured for fluorescence activity in a calibrated fluorometer (Optical Technology Devices, Elmsford, NY). The permeability-surface area (PS) product was calculated for fluorescein and FITC-dextran by multiplying the suffusate-to-plasma concentration ratio by the suffusate flow rate (25).

In a fourth series of experiments, a modified technique described by Baba et al. (2) was used to evaluate blood-brain barrier permeability in rabbits treated with TNBS (n = 8) or 50% ethanol vehicle (n = 7). Briefly, after the general preparation described above, 5% sodium fluorescein (mol wt 376) was given intravenously (2.5 ml/kg) and allowed to circulate for 30 min. Then an arterial blood sample (1 ml) was taken and an overdose of potassium chloride was administered intravenously to the anesthetized rabbit to cause death.

After the animal was confirmed to be dead, the brain was perfused by injecting 400 ml of saline into the left ventricle and aorta after ligation of the thoracic aorta below the tip of the cannula that was previously inserted through the femoral artery. Perfusion pressure was monitored and kept below 150 mmHg throughout this perfusion process. An absence of color in the effluent from the brain confirmed proper perfusion. The brain was removed from the skull, weighed, and homogenized in 40 ml of a 0.1 M borate buffer (pH = 10). Brain homogenate...
was centrifuged for 10 min at 1,200 g, and 1 ml of effluent was added to 4 ml of ethanol to precipitate proteins. The effluent and precipitated protein solution were centrifuged for 20 min at 15,000 g, and the supernatant was measured for fluorescence using a spectrophotometer. Plasma was extracted from the centrifuged blood sample that was taken just before death and diluted by a factor of 10,000.

The final tissue supernatant and diluted blood samples were then measured for fluorescent activity in a calibrated fluorometer (Optical Technology Devices). A standard curve for food and brain homogenate was plotted and used to measure the amount of fluorescein in each sample. The extraction ratio was calculated as the concentration of fluorescein in the brain (ng/g of tissue) divided by the concentration of fluorescein in the blood (ng/ml).

Measurement of cerebral blood flow. A fifth series of experiments measured cerebral blood flow with a radiolabeled microsphere technique (17) in TNBS-treated or untreated rabbits to determine whether acute colitis changes cerebral blood flow. In these experiments, animals were prepared as described in Preparation of animals, a thoracotomy was performed, and a cannula was placed in the left atrium for injection of microspheres (145Sc or 113Sn). An auxiliary artery was cannulated for withdrawal of a reference sample during the microsphere injection. Microspheres were mixed vigorously for at least 2 min and injected into the left atrium over a period of 15–20 s, and the catheter was flushed with heparinized saline.

Arterial blood was withdrawn (0.62 ml/min) from a femoral and axillary artery beginning 15 s before the injection of microspheres and continuing for 90 s after the flush (33). After the anesthetized rabbits were euthanized with an intravenous overdose of potassium chloride, the distal 10 cm of colon were removed for visual inspection. The brain was removed and fixed in Formalin. After at least 24 h, samples of brain were sectioned and weighed for counting. Reference blood samples and tissue samples were counted for 5 min in a Beckman G5500 gamma counter, and blood flow to the brain (Qf) was calculated as Qf = (Cf × Fb × 100)/CR, where Cf is counts per gram of tissue, Qb is reference blood flow, and CR is total activity (counts) of the reference blood sample (33).

Materials. L-Phenylephrine HCl, sodium fluorescein, and FITC-dextran were obtained from Sigma Chemical (St. Louis, MO). TNBS (1 M aqueous solution) was obtained from Fluka (Buchs, Switzerland) and lyophilized. Radiolabeled microspheres were purchased from NEN (Boston, MA).

Statistical analysis. Because of the heterogeneity of variance between groups, data from the first series of experiments (HAc vs. ethanol, permeability measurements) were compared at each time point using the Mann-Whitney test. Data from the second through the fifth series of experiments (TNBS vs. untreated and TNBS vs. 50% ethanol permeability experiments and blood flow experiments) were evaluated with an unpaired t-test. Data were first tested for homogeneity of variance with an F test (40). In cases where the variance of groups was significantly different, data were log-transformed and retested with an F test. An unpaired t-test with Welch’s approximation was used on data from the second, third, and fourth sets of experiments that had a significant difference in variances after log transformation (40). Damage scores (Fig. 1) for untreated, 50% ethanol-treated, and TNBS-treated rabbits were compared with scores of treated rabbits with a Kruskal-Wallis test (40). Damage scores for rabbits treated with 30% ethanol or HAc were compared with a Mann-Whitney test (40). The power (1-β) of each t-test that did not detect a statistically significant difference was calculated for a change of 20% and 50% from control (40). ANOVA

Fig. 1. Colon damage in untreated (n = 7) and animals treated with 50% ethanol (EtOH; n = 8), trinitrobenzene sulfonic acid (TNBS; n = 16), 30% EtOH (n = 8), or acetic acid (HAc) (n = 7) for 48 h, evaluated from criteria in Table 1. TNBS-treated animals had a significantly higher damage score than 50% EtOH-treated animals or untreated controls. HAc-treated animals had a significantly higher damage score than rabbits treated with its vehicle, 30% EtOH. Values are means ± SE. *P < 0.01 compared with control, with a Student-Newman-Keuls post hoc test was used to assess differences in mean arterial pressure. Changes in cerebrovascular resistance were evaluated with a t-test. Values are represented as means ± SE, and P < 0.05 was used for detection of significant differences between groups.

RESULTS

General effects of treatments. Forty-eight hours after treatment with HAc or TNBS, significant colonic inflammation was present that was not observed in either untreated or vehicle-treated animals (Fig. 1). Treatment with HAc, TNBS, or ethanol caused hyperemia in the distal colon that was visually apparent. In addition, colons treated with HAc or TNBS exhibited ulcerative lesions of the mucosa, some of which penetrated the outer muscular layers. Untreated animals had no colonic hyperemia or mucosal lesions. TNBS- and HAc-treated animals were typically lethargic and noticeably less active than control animals. Forty-three percent of HAc-treated rabbits and 64% of TNBS-treated rabbits exhibited fibrous peritoneal adhesions that were focused around the inflamed colon. Induction of colitis caused diarrhea in 71% and 64% of HAc- and TNBS-treated animals, respectively. None of the animals treated with 30% or 50% ethanol vehicle had adhesions. One animal treated with 50% ethanol exhibited diarrhea, and none of the 30% ethanol-treated animals had diarrhea. None of the untreated animals had diarrhea or adhesions.

Mean arterial pressure (MAP) varied between groups of experiments but in all cases was above the estimated lower limit of autoregulation of cerebral blood flow (50 mmHg; Ref. 11). In addition, MAP was similar in rabbits treated with HAc and 30% ethanol treatment alone (56 ± 3 and 53 ± 2 mmHg, respectively). In experiments that measured cerebral blood flow, there was no difference in MAP between untreated rabbits and rabbits treated with TNBS (76 ± 6 and 70 ± 4 mmHg, respectively). MAP was not different in animals in which blood-brain barrier permeability was measured as an extraction ratio. In these experiments MAP was 57 ± 2 mmHg in TNBS-treated rabbits and 70 ± 6 mmHg in 50% ethanol sham-treated rabbits. By con-
In other experiments where PS product was measured, MAP was greater in untreated rabbits than in TNBS-treated rabbits (83 ± 3 and 72 ± 3, respectively, P = 0.01).

Blood-brain barrier permeability and cerebral blood flow. Permeability of the blood-brain barrier to fluorescein was evaluated in HAc- and vehicle-treated rabbits. In these experiments, the PS product for fluorescein was calculated at 5-min intervals for 35 min (Fig. 2). In rabbits that were treated with HAc or vehicle, the PS product increased over the first 20 min following fluorescein administration. At this time, the PS product for fluorescein in rabbits treated with HAc was over eight times greater than that of vehicle-treated rabbits (2.7 ± 1.5 × 10⁻⁵ ml/s vs. 0.33 ± 0.05 × 10⁻⁵ ml/s).

Blood-brain barrier permeability was similarly evaluated in TNBS-treated and untreated rabbits by calculating the cerebrovascular PS product of fluorescein (Fig. 3A). In these experiments, the PS product with fluorescein was three times greater in animals with TNBS-induced colitis than that of untreated animals. The extraction ratio for fluorescein was also significantly increased by 75% in animals with TNBS-induced colitis when compared to vehicle-treated animals (Fig. 4).

FITC-dextran with an average molecular weight of ~71,000 was used to further define the characteristics of the changes in blood-brain barrier permeability that occur during acute experimental colitis. In these experiments, after 20 min of circulation, there were no significant differences in the PS product for FITC-dextran between TNBS-treated and control animals (Fig. 3B).

TNBS treatment did not have a significant effect on cerebral blood flow (CBF) in any area (Table 2). In addition, calculated cerebral vascular resistance was statistically similar in TNBS-treated and untreated control rabbits (Table 3).

**DISCUSSION**

This study examined the effects of experimental colitis on the permeability of the blood-brain barrier in rabbits. Our findings indicate that acute inflammation in the colon increases blood-brain barrier permeability for low-molecular-weight compounds such as fluorescein (mol wt 376). In contrast, blood-brain permeability for a much larger compound, FITC-dextran (mol wt 71,200), did not increase, indicating that the increase in permeability primarily affected extravasation of small molecules. In addition, colitis did not significantly alter cerebral blood flow or vascular resistance in the brain, suggesting that changes in PS product and extraction ratio were not due to an increase in vascular surface area (10). Blood-brain barrier permeability increased in two different models of colitis, which supports the concept that inflammation, and not the treatment used to induce inflammation, caused the observed increases in blood-brain barrier permeability. Similarly, blood-brain barrier permeability increased when two different methods of measurement were utilized, suggesting that the changes were not a result of the cranial window measurement technique.

**Fig. 2.** Blood-brain barrier permeability-surface area (PS) product for fluorescein in rabbits 48 h after vehicle treatment (n = 6) or induction of colitis with HAc (n = 6). Permeability measurements started 5 min after fluorescein infusion and were continued every 5 min for 35 min. Values are means ± SE. *P < 0.05, **P < 0.01 compared with EtOH at the same time point.

**Fig. 3.** Effects of TNBS treatment on blood-brain barrier permeability to fluorescein (A; mol wt 376) or FITC-dextran (B; mol wt 71,000) after circulating in the blood for 20 min. Power to detect a 20% change in the PS product to FITC-dextran was <0.30. A: for TNBS, n = 14; for control, n = 10. *P < 0.05 compared with control. B: for TNBS, n = 8; for control, n = 7.

**Fig. 4.** Effects of TNBS (n = 8) or vehicle (n = 7) treatment on blood-brain barrier permeability. Extraction ratio of fluorescein (mol wt 376) was measured after it circulated for 30 min. *P < 0.05 compared with vehicle control (ethanol).
CBF was not affected by colonic TNBS, and preliminary studies in our laboratory suggest that its vehicle, 50% ethanol, also has no effect. Similar studies confirm that 50% ethanol does not change blood flow in kidney, stomach, diaphragm, pancreas, adrenal glands, heart, tongue, masseter, latissimus, and bicep (unpublished observations).

In this study, PS product was used as an index of permeability (25). PS product values represent changes in permeability if the vascular surface area does not change. The brain is unique because virtually all of the capillaries are perfused continuously, and changes in flow result almost exclusively from changes in vascular diameter, not from capillary recruitment (15, 38). In fact, although the differences were not statistically significant, in our experiments colitis tended to decrease CBF and increase vascular resistance, which further argues against capillary recruitment and, therefore, an increase in surface area as the cause for increased PS product in these experiments.

MAP appeared to vary between some groups but in fact was similar in most of the treatment groups and their respective control groups. MAP was slightly different between the TNBS and the untreated control group when the PS product was measured. This difference was small, and in both cases MAP was well above the lower limit of autoregulation of CBF so a small difference in MAP would not have a significant effect on tissue blood flow or extravasation of tracer.

Although fluorescein is a relatively small molecule, it does not normally pass through the blood-brain barrier in significant amounts. In the present study, we detected an increase of 75–1100% in blood-brain barrier permeability to fluorescein in animals with acute experimental colitis. However, permeability of much larger molecules was restricted in our experiments. The findings indicate that the increase in blood-brain barrier permeability to fluorescein probably is not due to an increase in pinocytosis. An increase in endothelial pinocytotic activity gives rise to an increase in blood-brain barrier permeability to the same extent for both large and small sized molecules. In contrast, size-dependent increases in blood-brain barrier permeability suggest a loss of tight junction integrity between endothelial cells (25). Our findings, therefore, suggest that inflammation in the colon can cause an increase in blood-brain barrier permeability for small molecules only, which results from increases in the size of the clefts or pores between endothelial cells (25).

Colitis may affect blood-brain barrier permeability through several different mechanisms. For example, inflammation in the colon may activateafferent nerve fibers projecting to the brain and produce a large release of vasoactive neurotransmitters that could cause

Table 2. Regional CBF in control animals and animals with experimental colitis

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Control CBF, ml·min⁻¹·100 g⁻¹</th>
<th>n</th>
<th>48-h TNBS CBF, ml·min⁻¹·100 g⁻¹</th>
<th>n</th>
<th>P Value 20%</th>
<th>P Value 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choroid plexus of lateral ventricle</td>
<td>435 ± 48</td>
<td>7</td>
<td>320 ± 57</td>
<td>8</td>
<td>0.09 0.29</td>
<td>0.77 0.81</td>
</tr>
<tr>
<td>Choroid plexus of 4th ventricle</td>
<td>295 ± 31</td>
<td>5</td>
<td>251 ± 33</td>
<td>7</td>
<td>0.24 0.30</td>
<td>0.81 0.81</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>92 ± 13</td>
<td>7</td>
<td>70 ± 4</td>
<td>8</td>
<td>0.09 0.31</td>
<td>0.89 0.89</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>112 ± 20</td>
<td>7</td>
<td>92 ± 5</td>
<td>8</td>
<td>0.26 0.29</td>
<td>0.78 0.78</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>110 ± 14</td>
<td>7</td>
<td>91 ± 7</td>
<td>8</td>
<td>0.17 0.32</td>
<td>0.92 0.92</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>52 ± 7</td>
<td>7</td>
<td>42 ± 3</td>
<td>8</td>
<td>0.15 0.32</td>
<td>0.92 0.92</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>65 ± 4</td>
<td>7</td>
<td>59 ± 4</td>
<td>8</td>
<td>0.25 0.51</td>
<td>0.99 0.99</td>
</tr>
<tr>
<td>Caudateothalamus</td>
<td>60 ± 6</td>
<td>7</td>
<td>54 ± 3</td>
<td>8</td>
<td>0.31 0.40</td>
<td>0.99 0.99</td>
</tr>
<tr>
<td>Midbrain</td>
<td>70 ± 5</td>
<td>7</td>
<td>61 ± 3</td>
<td>8</td>
<td>0.08 0.60</td>
<td>0.99 0.99</td>
</tr>
<tr>
<td>Pons</td>
<td>50 ± 7</td>
<td>7</td>
<td>44 ± 3</td>
<td>7</td>
<td>0.41 0.31</td>
<td>0.88 0.88</td>
</tr>
<tr>
<td>Medulla</td>
<td>58 ± 10</td>
<td>7</td>
<td>52 ± 5</td>
<td>7</td>
<td>0.64 0.28</td>
<td>0.67 0.67</td>
</tr>
</tbody>
</table>

Cerebral blood flow (CBF) values are means ± SE; n = no. of animals. P values are for 48-h trinitrobenzene sulfonic acid (TNBS) treatment vs. control. Power values correspond to detection of a change of 20% or 50% from control.

Table 3. Regional CVR in control animals and animals with experimental colitis

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Control CVR, mmHg·min·ml⁻¹·100 g⁻¹</th>
<th>n</th>
<th>48-h TNBS CVR, mmHg·min·ml⁻¹·100 g⁻¹</th>
<th>n</th>
<th>P Value 20%</th>
<th>P Value 50%</th>
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<tbody>
<tr>
<td>Choroid plexus of lateral ventricle</td>
<td>0.18 ± 0.01</td>
<td>7</td>
<td>0.26 ± 0.04</td>
<td>8</td>
<td>0.10 0.12</td>
<td>0.48 0.48</td>
</tr>
<tr>
<td>Choroid plexus of the fourth ventricle</td>
<td>0.28 ± 0.04</td>
<td>5</td>
<td>0.30 ± 0.04</td>
<td>7</td>
<td>0.68 0.45</td>
<td>0.68 0.68</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.88 ± 0.07</td>
<td>7</td>
<td>1.01 ± 0.07</td>
<td>8</td>
<td>0.16 0.37</td>
<td>0.98 0.98</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>0.78 ± 0.11</td>
<td>7</td>
<td>0.78 ± 0.06</td>
<td>8</td>
<td>0.97 0.30</td>
<td>0.75 0.75</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>0.73 ± 0.06</td>
<td>7</td>
<td>0.80 ± 0.08</td>
<td>8</td>
<td>0.48 0.34</td>
<td>0.87 0.87</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.55 ± 0.13</td>
<td>7</td>
<td>1.72 ± 0.17</td>
<td>8</td>
<td>0.45 0.32</td>
<td>0.85 0.85</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.17 ± 0.10</td>
<td>7</td>
<td>1.23 ± 0.13</td>
<td>8</td>
<td>0.73 0.35</td>
<td>0.88 0.88</td>
</tr>
<tr>
<td>Caudateothalamus</td>
<td>1.13 ± 0.12</td>
<td>7</td>
<td>1.33 ± 0.13</td>
<td>8</td>
<td>0.87 0.34</td>
<td>0.83 0.83</td>
</tr>
<tr>
<td>Midbrain</td>
<td>1.10 ± 0.11</td>
<td>7</td>
<td>1.18 ± 0.10</td>
<td>8</td>
<td>0.63 0.60</td>
<td>0.99 0.99</td>
</tr>
<tr>
<td>Pons</td>
<td>1.61 ± 0.17</td>
<td>7</td>
<td>1.66 ± 0.21</td>
<td>7</td>
<td>0.86 0.31</td>
<td>0.88 0.88</td>
</tr>
<tr>
<td>Medulla</td>
<td>1.43 ± 0.14</td>
<td>7</td>
<td>1.44 ± 0.18</td>
<td>7</td>
<td>0.97 0.28</td>
<td>0.67 0.67</td>
</tr>
</tbody>
</table>

Cerebrovascular resistance (CVR) values are means ± SE; n = no. of animals. P values are for 48-h TNBS treatment vs. control. Power values correspond to detection of a change of 20% or 50% from control.
an increase in cerebrovascular permeability. It is also possible that factors responsible for increasing permeability of the blood-brain barrier during colitis are transported from the inflamed colon to the brain via the circulatory system. For example, there is an increase in the amount of circulating TNF-α in patients with inflammatory bowel disease, and TNF-α can cause an increase in blood-brain barrier permeability (8, 21, 24). Mahmud et al. demonstrated that microalbuminuria (increased renal vascular permeability) was correlated to the extent of IBD activity and TNF-α levels in the blood of these patients (22, 23). Immune cells, particularly neutrophils, are suspected of being the messenger system in this humoral mechanism (5). Another possible mechanism for increases in blood-brain barrier permeability in acute colitis may be bacteria or bacterial endotoxin infiltration through the inflamed colon, leading to endotoxemia (30). The unknown mechanisms responsible for increasing the permeability of the pulmonary and renal vasculature during colitis may also be responsible for increases in blood-brain barrier permeability seen in this study (14, 22, 23, 27).

The results of the present study may have important implications for the pharmacological treatment of patients with inflammatory bowel disease. Large hydrophilic drugs do not normally enter the brain when the blood-brain barrier is intact (32). However, our studies suggest that some drugs may have greater entry into the central nervous system with more pronounced effects in patients with IBD because of an increase in blood-brain barrier permeability.

Increased blood-brain barrier permeability may also permit endogenous circulating hormones to reach the brain in an uncontrolled fashion. For example, leptin is a relatively small protein (mol wt ~16,000) involved in the signaling of hunger to the brain. Normally, leptin enters the brain via a saturable, specific transport mechanism (6). An increase in blood-brain barrier permeability may increase the amount of leptin that enters the brain, which would decrease hunger. Anorexia is common in patients with colitis (20), and data from the present study provide evidence to support the concept that an increase in blood-brain barrier permeability to leptin may be a contributing factor in this phenomenon.

In summary, our studies in two separate models of experimental colitis demonstrate that colitis causes an increase in blood-brain barrier permeability. In addition, the increase in PS product is unlikely to be caused by an increase in surface area because there is very little capillary recruitment in the brain, and neither CBF nor cerebral vascular resistance was changed as a result of the development of colitis. Our findings suggest that the increase in blood-brain barrier permeability is due to the opening of endothelial tight junctions and not to an increase in transcellular transport (25). These findings also demonstrate that inflammation outside of the brain can have effects on the permeability of the blood-brain barrier, which may have implications on the progression and treatment of this disease process.

This study was supported in part by grants from the Crohn’s and Colitis Foundation of America and the National Science Foundation (NSF) under grant OSR-9452894, and the South Dakota Futures Fund through the South Dakota NSF-EPSCoR Program.

Address for reprint requests and other correspondence: J. L. Williams, Dept. of Physiology and Pharmacology, School of Medicine, The University of South Dakota, 414 E. Clark St., Vermillion, SD 57069 (E-mail: jwilliam@usd.edu).

Received 25 June 1998; accepted in final form 22 January 1999.

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