Selective Extravascular Escape of Albumin into the Cerebral Cortex of the Diabetic Rat

WILLIAM T. STAUBER, SHU-HUI ONG, AND ROBERT S. McCUSKEY

SUMMARY

The extravasation of plasma proteins (albumin, IgG, and complement C_3) into the cerebral cortex was studied in streptozotocin-induced diabetic rats using immunohistochemical techniques. The results indicate that albumin, but not IgG or complement C_3, selectively enters the cerebral cortex within 2 wk after induction of diabetes. It is suggested that albumin may be an oncotic substance that contributes to diabetic cerebral microangiopathy, astrocytic swelling, and the cerebral edema that occasionally is seen during fluid and insulin therapy of juvenile ketoacidotic diabetes.

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The central nervous system responds to peripheral hyperosmolality and dehydration by the accumulation of osmotically active substances. These molecules attract water and help the brain resist changes in total brain water content and volume. The generation of "idiogenic" osmoles, which permit rapid reestablishment of normal brain water content, were reported in diabetic rabbits in the face of peripheral dehydration and hyperosmolality.[1] If this adaptation of the central nervous system to peripheral dehydration and hyperosmolality occurs by the accumulation of oncotic molecules that selectively cross endothelial cells, then overhydration of the brain during fluid and insulin therapy would occur if there is a slow reverse transport of these molecules. Evidence to support such a hypothesis exists and central nervous tissue subjected to the peripheral dehydration and hyperosmolality of diabetes tended toward overhydration during fluid and insulin therapy.[2] Clinically, ketoacidotic juvenile diabetics occasionally die of uncontrolled cerebral edema after fluid and insulin therapy;[3] and recent studies have demonstrated cerebral edema in streptozotocin-induced diabetic rats a few hours after initiating insulin and fluid therapy.[4]

The purpose of this study was to investigate by immunohistochemical techniques the nature of this oncotic molecule that we believed might be derived from the blood. The results reported in this paper indicate that albumin, but not other serum proteins, increase in the cerebral cortical tissue of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats weighing 300–350 g were used. One group of animals was made diabetic with a single i.p. injection of streptozotocin (75 mg/kg).[5–7] Animals were selected for these immunohistochemical studies if, 2 wk after streptozotocin, they demonstrated blood glucose values of greater than 300 mg/dl and were ketoacidotic. A few longer term diabetic rats (5–6 mo) were also evaluated; these were not ketoacidotic. Vehicle-treated control animals of similar age and sex were also evaluated. In addition, a group of streptozotocin-treated animals was administered lente insulin (U6/100/g, s.c., Eli Lilly Co.) daily beginning 24 h after being administered streptozotocin. Only animals maintaining plasma glucose levels of 90–180 mg/dl were selected for study. Spontaneously hypertensive (SHR) and normotensive (Wistar-Kyoto) rats (Charles River Co.) of comparable age were also studied for comparison.

Under sodium pentobarbitol anesthesia (25 mg/kg), a portion of the skull was removed to expose one hemisphere of the cerebral cortex without disrupting the superior dural sinus. This cortical area was gently rinsed with warm saline and a piece of brain was quickly excised, placed on a cork with O.C.T. compound (Miles Laboratories, Inc.) surrounding it, and immediately frozen in isopentane cooled by liquid nitrogen. Cryosectioning was then performed at −40°C on a Bright cryostat (Model FS/FAS/M/LT, Bright Instruments Co.) and resulting 4-μm sections were mounted on glass slides and allowed to air dry.

Indirect immunofluorescent examination of frozen tissue sections[9] of rat cerebral cortex with fluorescein-labeled second antibody was performed by methods of Ong et al.[10]

From the Departments of Physiology and Anatomy, School of Medicine, West Virginia University, Morgantown, West Virginia 26506.
Address reprint requests to William T. Stauber at the above address.
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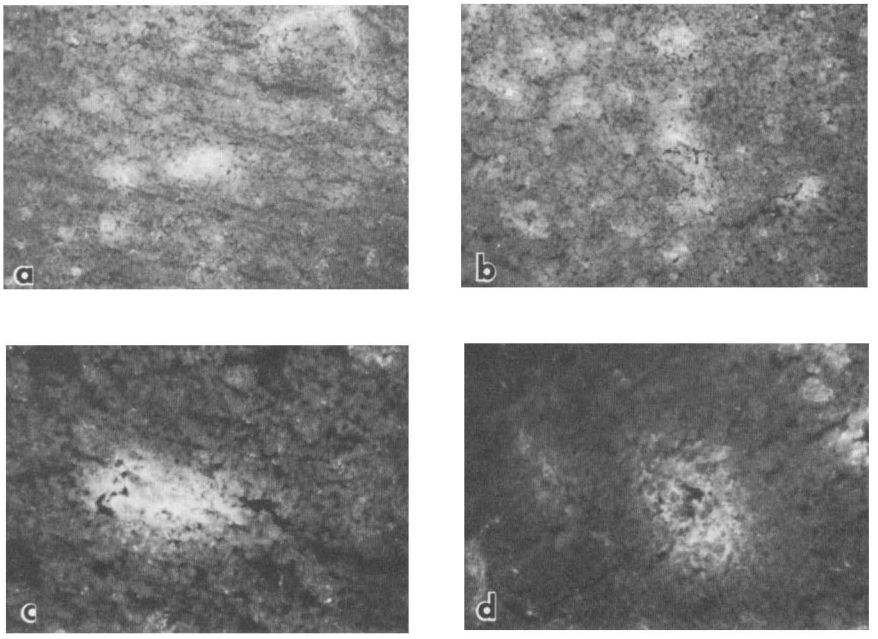

FIGURE 1. Immunohistochemical localization of albumin in cerebral cortical tissue: 2-wk diabetic rats. a and b, 150×; c and d, 240×.

Briefly, the slides were washed three times for 3 min each with 0.01 M sodium phosphate in 0.9% NaCl (pH 7.4) (PBS) before staining. Excess PBS was removed before adding specific antibody. A drop of a 1:30 dilution of specific goat antirat antisera against albumin, IgG, or complement C_3 (Cappel Laboratories, Inc.) was applied to the slide. Slides then were placed in the moist chamber. After 30 min, the slides were removed, antisera were rinsed off, and the slides were placed in a staining dish followed by three rinses with PBS for 3 min each. Then, a second antisera, rabbit-antigoat IgG tagged with FITC (Miles Laboratories, Inc.) at 1:30 dilution was applied to the tissue for 30 min. At the end of 30 min, slides were removed and again washed three times for 3 min each with PBS. Next, using a mounting solution of 50% PBS and 50% glycerin, coverslips were applied and finally the slides were examined with a Leitz-Orthoplan fluorescence microscope. To demonstrate the specificity of the procedure, normal goat or normal rat serum was applied to some slides, but failed to produce any significant staining.

Method specificity and antibody specificity for the immunohistochemical reactions were determined according to the criteria of reliability established by Petrusz et al.[8] Briefly, serial dilutions of antisera established the concentration of antisera necessary for positive staining but which eliminated all background staining. Nonspecific reactions were "blocked" by incubation in the presence of 1% normal rabbit serum for 15 min followed by a brief wash in PBS before the application of specific primary antisera. The latter experiments were not different from those presented in this

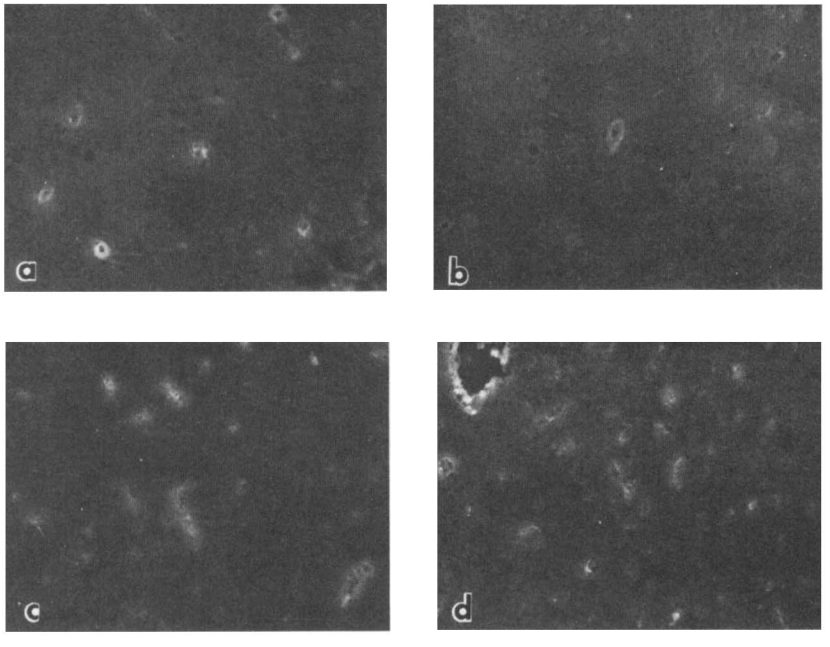

FIGURE 2. Immunohistochemical localization of albumin in cerebral cortical tissue: 2-wk diabetic rats treated with insulin, a and b, 150×; vehicle-treated controls, c and d, 150×.

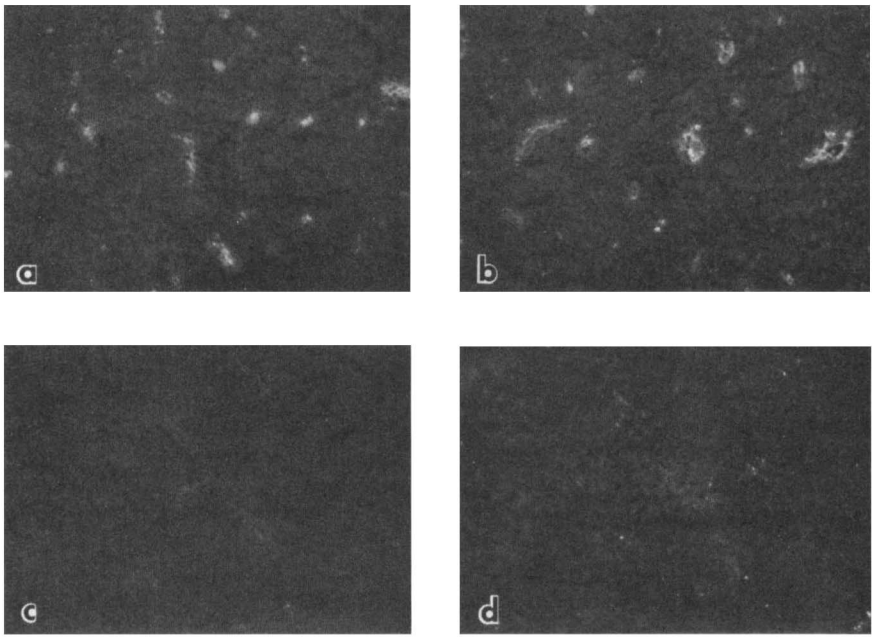

FIGURE 3. Immunohistochemical localization of serum proteins in cerebral cortical tissue. (a) IgG, 2-wk diabetic; (b) IgG, control; (c) normal goat serum, 2-wk diabetic; and (d) normal goat serum, control. a and b, 150×; c and d, 240×.

paper. In addition, antibody specificity was tested by immunodiffusion, resulting in a single preciptin line against whole serum and absorption with purified antigen or whole rat serum. The absorption studies were performed before staining, and staining could be eliminated at a concentration of 1 mg/ml albumin per 8 mg/ml of antisera or 1 mg/ml of whole rat serum per 8 mg/ml of antisera after the antigen-antibody complexes were removed by centrifugation (2800 rpm × 30 min). At a concentration of 100 μg/ml, about 40% of the albumin staining disappeared.

A few experimental trials were initiated using direct labeled primary antibody. The staining pattern was identical to that presented in this paper but the intensity was much reduced. Therefore, all results presented in this communication represent indirect antibody histochemical techniques. All antibodies and normal goat serum were applied to sections from the same tissue samples and processed on the same day. These procedures, plus identical photography settings, have allowed valid comparisons of antigen localizations.

RESULTS

The immunohistochemical localization of albumin, IgG, and complement C_3 was demonstrated in the cortex of diabetic and normal rats. Brains from eight diabetic animals were all positive for albumin in cortical tissue outside the vascular network (Figures 1a–d) but none of the vehicle-treated (Figures 2c and d) or insulin-treated controls (Figures 2a and b) were positive. In short-term (2 wk) diabetic animals, fluorescence appeared as intense halos around many vessels (Figures 1c and d) and in patches throughout about 75% of the cortex (Figures 1a and b). These areas appeared to increase in size and density with the duration of the diabetic state up to 6 wk, and then no further increases could be detected. In contrast, IgG (Figures 3a and b) and complement C_3 (not shown) remained inside blood vessels in both diabetic and normal animals and thus served as a control for hemorrhage should that occur. In animals that were handled properly, no hemorrhage was detected in either control or diabetic animals.

In similar experiments using spontaneously hypertensive rats, no differences were observed between the hypertensive rats and the vehicle-treated, nondiabetic control rats presented in Figures 2 and 3 for albumin, IgG, or complement C_3 (not shown). No nonspecific staining was observed from sections treated with normal goat serum (Figures 3c and d).

DISCUSSION

Two weeks after streptozotocin, a significant increase in the density of the cerebral cortex has been observed in diabetic animals similar to those used in this study.[11] The change in brain water content was hypothesized to be associated with an increase in brain solids rather than a loss of brain water. The results in this study support such a concept and at least one such specific solid could be identified as that of albumin.

Transcapillary escape of albumin and IgG increased in patients with short- and long-term juvenile diabetes.[12,13] Using ^{125}I-albumin, transcapillary escape rates were calculated as that fraction of intravascular mass that can pass into the "extravascular space" per unit of time. The difficulty with interpretation of these studies is the definition of what constitutes the "extravascular space." The presence of albumin but not IgG in cerebral cortical tissue would suggest a heterogeneity in "extravascular space" to which different molecules might escape.

Similarly, transcapillary escape was increased in patients with essential hypertension.[14] In fact, both hypertensive and diabetic patients had transcapillary escape rates of albumin that were increased by approximately 30% from controls.[12] During acute hypertension, radioiodinated albumin has also been reported to cross the blood-brain barrier.[15] However, using immunohistochemical methods in spontaneously hy-

pertensive rats, no change was found in the extravascular location of albumin when compared with normotensive animals. An inherent problem in interpretation of these apparently conflicting results stems from a lack of understanding of what constitutes the blood-brain barrier. If the functional blood-brain barrier lies in the extravascular space, perhaps at the level of the basement membrane and/or even astrocytes, then increased albumin could be present in brain samples of hypertensive animals but not in the neuropil. In marked contrast, albumin in the diabetic rats was present in cerebral cortical tissue (neuropil) at some distance from the vessels. However, if special care is taken to select stroke-prone subclasses of spontaneously hypertensive rats, 53% of these rats will demonstrate albumin leakage.[16] These observations may be due in part to hemorrhage rather than selective loss of albumin such as is seen in the present study where, to our knowledge, there was no evidence of hemorrhage at the time of the experiments.

Presumably, edema does not result from the extravasation of albumin during diabetes mellitus because of a hyperosmolar vascular compartment.[17] Perhaps it is necessary to define this situation as extracellular dry edema because insulin therapy and restoration of fluid to the vascular compartment lead to brain swelling.[4] Therefore, the selective transfer of albumin across the blood-brain barrier appears to be a unique pathologic response of the uncontrolled diabetic state.

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