Acta Neuropathol (Berl) (1985) 68:122–129

Acta Neuropathologica

The Biphasic Opening of the Blood-Brain Barrier to Proteins Following Temporary Middle Cerebral Artery Occlusion

T. Kuroiwa, P. Ting, H. Martinez, and I. Klatzo

Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bldg. 36, Rm. 4D04, Bethesda, MD 20205, USA

Summary. The behavior of the blood-brain barrier (BBB) was studied in cats following release after 1-h middle cerebral artery (MCA) occlusion. The regional cerebral blood flow (rCBF) was determined by hydrogen clearance method in the caudate nucleus and the cerebral cortex. The BBB was assayed with Evans blue (EB) tracer and by immunohistochemical peroxidase-antiperoxidase (PAP) method. Following release of MCA occlusion, there were two openings of the BBB, separated by a refractory period. The first opening, occurred shortly after recirculation; this was associated with rCBF below 15 ml/100 g/min during the ischemic period and a pronounced reactive hyperemia promptly following release of MCA occlusion. A refractory period of the BBB was indicated by the absence of EB leakage in cats injected with the tracer 30 min before killing at 3 h after recirculation, although the rCBF values in these animals were even lower (6 ± 1 ml/100 g/min) during occlusion, and all of them showed a pronounced hyperemia after recirculation. The occurrence of the previous BBB opening in these animals was confirmed by the PAP staining. The second opening of the BBB was observed at 5 and 72 h after recirculation in cats which were injected with EB 30 min before killing, and which showed rCBF below 15 ml/100 g/min during occlusion, followed by a pronounced reactive hyperemia. No EB extravasations were observed at any time in cats in which the rCBF during occlusion was above 15 ml/100 g/min and which failed to show a marked reactive hyperemia.

Key words: Cerebral ischemia – Blood-brain-barrier – Cerebral blood flow – Reactive hyperemia

Offprint requests to: Dr. Igor Klatzo (address see above)

Introduction

It can be assumed, in accordance with the concept of the BBB as a number of systems regulating the passage of various types of substances, that in pathologic conditions these systems can be unequally affected and, indeed, selective features in abnormal permeability of the barrier have been described in cerebral ischemia (Juhler et al. 1984; Klatzo 1983).

With regard to protein tracers, a remarkable resistance of the BBB to ischemic injury has been brought out by Broman (1949), and a prevailing opinion has been that the BBB breakdown to proteins occurs only in association with ischemic infarction (Olsson et al. 1971; Petito et al. 1982). In studies of this laboratory, it has been shown that BBB damage in ischemia may become evident after a definite delay, the length of which appears to be related to severity of ischemic insult, according to the principle of the maturation phenomenon (Ito et al. 1975; Fujimoto et al. 1977; Klatzo 1975). On the other hand, a prompt BBB breakdown to proteins has been demonstrated in cerebral ischemia associated with embolism (Nishimoto et al. 1978).

The mechanism of passage of macromolecular substances, such as proteins, across the endothelial barrier in pathologic conditions has been hotly debated, the main controversy being focused on the respective roles of pinocytotic transport vs., opening of the interendothelial tight junctions (Cervos-Navarro et al. 1983). Although the involvement of the former has been described in a majority of pathologic conditions associated with BBB leakage, the presence of the latter has been stressed in breakdown of the BBB to proteins in hyperosmotic conditions (Nagy and Huttner 1983). The third mechanism implied in leakage of proteins has been considered to be related to acute destruction

of the endothelium, occurring within an area of necrotic infarction.

The question of the BBB behavior in cerebral ischemia has significant clinical implications, since the abnormal entry of serum proteins into brain tissue introduces the element of vasogenic edema, which can considerably influence the dynamics of ischemic injury (Kuroiwa et al. 1985; Klatzo 1985).

Stimulated by our recent observations indicating that a release of arterial occlusion can be followed by two, distinctly separated in time openings of the barrier to proteins (Suzuki et al. 1983), the present study was undertaken to elucidate further the conditions responsible for the BBB disturbance in cerebral ischemia.

Materials and Methods

Thirty-six cats of both sexes with a body weight between 2.5 and 4.0 kg were subjected to two separate procedures, 1 week apart.

The First Procedure

Ketamine (10 mg/kg) and xylazine (2 mg/kg) were injected i.m. initially for tracheal intubation. The cats were then given 2% halothane-50% nitrous oxide-50% oxygen by means of an Ohio unitrol anesthesia apparatus. Under strict aseptic techniques femoral artery and vein were cannulated for monitoring arterial blood pressure, hematocrit values, and blood gases. The free ends of the catheters were guided s.c. and secured on the back of the animal. Rectal temperature was recorded continuously.

The cat's head was then positioned in a David Kopf model 1504 stereotaxic apparatus. Under sterile conditions, the proximal part of the left middle cerebral artery (MCA) was exposed through a transorbital approach, carefully freed from the arachnoid membrane, and a 6-0 Prolene thread was looped around the artery close to its origin. The free ends of the thread were gently passed through two small holes (2 mm apart) of a small polyvinyl plate, placed loosely on the MCA. The thread was then passed within a polypropylene tube which was anchored to the orbit by dental cement after the craniotomy opening was sealed initially with gelfoam (Fig. 1). The skin incision over the orbit was then sutured.

Platinum microelectrodes were inserted stereotaxically, according to coordinates from the Snider's atlas for cats, into the caudate nucleus and the cortex of the ectosylvian gyrus, supplied by the MCA. In some cats of each group cortical and caudate recordings were made also on the opposite side. The sensing microelectrode was made of epoxylite-insulated platinum wires with a bared tip of 0.5 mm length and 50 μm in diameter. The tip was electrolytically coated with platinum black to increase surface area. A left and right reference miniature stainless steel plate screws were drilled into parieto-occipital bone. The electrodes were anchored permanently on the cranium by means of dental acrylic resin (Haining et al. 1968). The animals were returned to their cages, and antibiotic (Flocillin) was given daily for 5 days.

Second Procedure

Seven days after the first surgery, a cat was given 10 mg/kg pentobarbital i.p. and placed in a specially designed plexiglass cat box (Romano et al. 1980). The rCBF was determined by a standard inhalation hydrogen clearance technique.

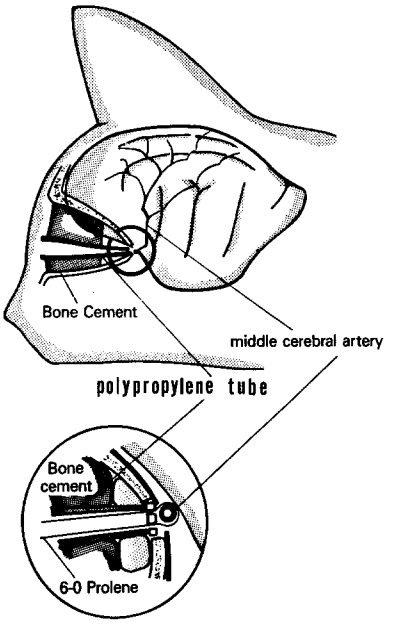


Fig. 1. Diagram of the procedure with the device used for the MCA occlusion

The electric circuit used to read the currents generated by hydrogen-platinum oxidation was described in detail by others (Young 1980; Fein et al. 1975; Senter et al. 1978). The output was displayed on a Gould 8-channel polygraph with individual baseline setting and offset balance control. The platinum microelectrode was polarized at +600 mV. Hydrogen (40%)-oxygen (30%) gas mixture was given to the cat through a plexiglass head chamber with ambient oxygen continuously monitored and kept at 30%. The duration of hydrogen inhalation varied between 7 and 15 min so as the minimize uneven tissue compartments saturation (Pasztor et al. 1973). The first 40 s of hydrogen clearance was excluded for possible hydrogen recirculation (Halsey et al. 1977). The rCBF was calculated from the equation: $F = \lambda\,(0.693/T^1/_2) \times 100$, where F = flow in ml/100 g/min; λ = brain/blood partition coefficient for hydrogen which is 1; 0.693 = natural logarithm of 2; $T^1/_2$ = half time of desaturation in minutes (Aukland et al. 1964). For statistical analysis, the unpaired Student's t-test was used and the results expressed as mean ± SEM.

Control baseline values of rCBF, arterial blood pressure, and blood gases and hematocrit values were recorded. The cat was then briefly and lightly anesthetized with an oxygen-halothane mixture, and the skin over the left orbit was infiltrated with 2% xylocaine to carry out the incision to expose the thread, which was looped around the MCA. Ischemia was produced by traction on the thread for 1 h; the release of occlusion was accomplished by removing the thread. The effect of leakage of CSF, which may affect the intracranial pressure, was minimized by sealing the occlusion device immediately after thread manipulations. Arterial blood pressure was monitored continuously, and blood gases and hematocrit values were determined with each rCBF measurement. Rectal temperatures were recorded continuously during day 1 of the experiment.

To evaluate the blood-brain-barrier (BBB), 5 ml 2% Evans blue (EB) was injected i.v. at various times following recirculation. Passage of the tracer into brain parenchyma was assessed

Table 1. Mean arterial blood pressure, pH, pO_2, pCO_2 and hematocrit values

	Baseline	Occlusion	15 min	30 min	1 h	2 h	3 h	4 h	5 h
MABP	140	132	128	129	131	127	127	123	127
(mm Hg)	±4	±2	±3	±3	±3	±3	±3	±5	±4
pO_2	128	123	132	129	127	121	131	111	114
(mm Hg)	±4	±4	±4	±4	±3	±4	±7	±8	±8
pCO_2	30.2	28.8	28.6	27.8	28.8	28.0	28.0	27.0	27.6
(mm Hg)	±0.5	±0.5	±0.6	±0.6	±0.6	±0.4	±0.7	±1.1	±0.6
pH	7.377	7.380	7.366	7.377	7.376	7.384	7.384	7.393	7.399
	±0.001	±0.008	±0.009	±0.007	±0.008	±0.006	±0.011	±0.014	±0.015
Hct (%)	29.6	29.9				28.0			
	±0.7	±0.6				±0.5			

Values expressed as Mean ± SEM

by visual inspection of the paraformaldehyde perfused coronal blocks of the brains, sectioned in the vicinity of the electrode insertions. In addition, frozen sections from several brains were examined under the fluorescence microscope to observe microscopically extravasations of the EB, which fluoresces brightly red under U.V. light.

Concerning the rCBF recordings, the values on the side of occlusion above 30 ml/100 g/min during 1 h ischemia were either above thresholds for ischemic tissue injury, or they were related to improper positioning of the electrodes, and therefore such recordings were excluded from the study. Four groups of animals with different recirculation time were studied.

Group I consisted of nine cats which were injected with EB immediately following release of occlusion and killed after 2 h. The evaluation of the relationship between BBB behavior and rCBF changes in this group was based on recordings from 13 locations in ischemically affected brain tissue. In addition to baseline values, the rCBF in group I was measured during occlusion, at 15-min, 30-min, 1-hr, and 2-h intervals following recirculation.

Group II comprised 11 cats, which were injected with EB following 2.5-h recirculation and killed 30 min later. The rCBF was measured from 16 ischemic sites at intervals as above, with additional rCBF evaluation at 3 h.

In *group III* consisting of ten cats, killed 5 h after recirculation and EB injection 30 min before killing, the rCBF determinations were extended up to 5 h and included recordings on the occluded side from 15 ischemic sites.

In *group IV*, six cats were killed after 3 days of recirculation with EB injection 30 min before killing. After rCBF measurement at 5 h, the animals were returned to their cages. In these cats, rCBF was measured also at 10 h, 1, 2, and 3 days and includes recordings from six ischemic sites.

The cats were killed by injection of pentobarbital followed by transcardiac perfusion with 4% paraformaldehyde. The brains were fixed in similar solution for 1 week and then cut coronally at the levels of electrode insertion sites and photographed. The coronal blocks were embedded in paraffin, and serial 20-μm sections were stained alternately with hematoxylin-eosin (HE) and cresyl violet for histological examination and electrode tip verification.

The peroxidase-antiperoxidase (PAP) method for demonstration of extravasated serum proteins was carried out on paraffin-embedded sections from the five cats in group II as follows: after deparaffinizing and hydrating, the sections were subjected to 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity. Following washing in 0.3% Triton X-100 for 30 min and in 0.02 M, pH 7.6, phosphate-buffered saline (PBS) for 15 min, the sections were subjected to the following incubations, between which they were briefly rinsed with PBS: (1) in normal goat serum, diluted 1:15 in PBS for 1 h, (2) in diluted 1:1,000 rabbit anti-cat serum, overnight at 4° C, (3) in goat anti-rabbit serum, diluted 1:40 for 30 min at room temperature, and (4) in PAP-rabbit complex, diluted 1:100 in PBS, 30 min at room temperature. The reaction to visualize peroxidase activity was carried out by immersing the sections in 0.01% hydrogen peroxidase and 0.05% 3,3′-diamino-benzidine-tetrahydrochloride in 0.1 M Tris buffer for 2–5 min. For control of specificity of immunocytochemical staining, the normal rabbit serum was used instead of rabbit anti-cat serum, or by substituting for rabbit anti-cat serum a supernatant of this serum obtained after absorbing anti-cat antibodies with normal cat serum and removing the antigen-antibody complex by centrifugation.

Results

The animals tolerated the surgical procedures well. The systemic arterial blood pressure and blood gases showed no significant changes before, during or after the MCA occlusion (Table 1). The rCBF on the opposite side revealed no significant changes (Table 3). The cats kept for 3 days showed no mortality, but revealed clinical signs characteristic of an ischemic lesion in the left hemisphere.

Concerning the evaluation of the BBB with the EB tracer, the placement of the electrodes could be occasionally recognized both in the ischemic and in the contralateral, non-ischemic hemispheres as narrow bands of brown or blue discoloration, outlining the electrode tracks. The more extensive, irregular shaped EB extravasations were sometimes observed in both hemispheres in the cerebral cortex at the site of an electrode penetration into brain tissue and occasionally extending into the underlying white matter. Another form of a traumatic, non-ischemic EB leakage was commonly seen in the basal regions of the left hemisphere, adjacent to the site of surgical trauma associated with the transorbital MCA occlusion.

Table 2. rCBF in ml/100 g/min on the side of MCA occlusion

		Base-line	Occlu-sion	Recirculation										
				15 min	30 min	1 h	2 h	3 h	4 h	5 h	10 h	24 h	48 h	72 h	BBB
Group I	A(5)	131 ± 15	8 ± 2*	370 ± 88	352 ± 63*	300 ± 64	286 ± 108								+
	B(8)	113 ± 30	17 ± 3	166 ± 58	162 ± 50	208 ± 63	152 ± 43								−
Group II	(16)	114 ± 14	6 ± 1	482 ± 82	372 ± 79	291 ± 58	241 ± 44	251 ± 51							−
Group III	A(8)	128 ± 32	9 ± 2*	284 ± 49	285 ± 35	304 ± 42	321 ± 44*	303 ± 55	267 ± 40	186 ± 41					+
	B(7)	104 ± 10	16 ± 2	194 ± 45	182 ± 44	199 ± 49	185 ± 32	215 ± 42	201 ± 47	208 ± 60					−
Group IV	(6)	100 ± 32	9 ± 2	283 ± 41	228 ± 43	240 ± 71	249 ± 57	189 ± 39	163 ± 31	120 ± 24	80 ± 15	106 ± 21	106 ± 31	100 ± 40	+

The number of recorded sites is shown in parenthesis
* $P < 0.05$ (comparison within each subgroup)

Table 3. rCBF in ml/100 g/min (contralateral, non-ischemic side)

Group	Base-line	Occlu-sion	Recirculation										
			15 min	30 min	1 h	2 h	3 h	4 h	5 h	10 h	24 h	48 h	72 h
I. (13)	110 ± 10	95 ± 9	103 ± 10	108 ± 11	102 ± 10	87 ± 8							
II. (6)	132 ± 29	116 ± 25	127 ± 28	151 ± 45	147 ± 32	145 ± 35	153 ± 38						
III. (9)	114 ± 22	106 ± 29	97 ± 20	96 ± 18	100 ± 16	104 ± 17	109 ± 21	106 ± 18	110 ± 16				
IV. (12)	146 ± 21	120 ± 15	127 ± 17	114 ± 20	107 ± 12	129 ± 21	128 ± 21	118 ± 16	117 ± 19	112 ± 22	119 ± 23	146 ± 28	125 ± 30

Values expressed as Mean ± SEM
Number of sites indicated in parenthesis

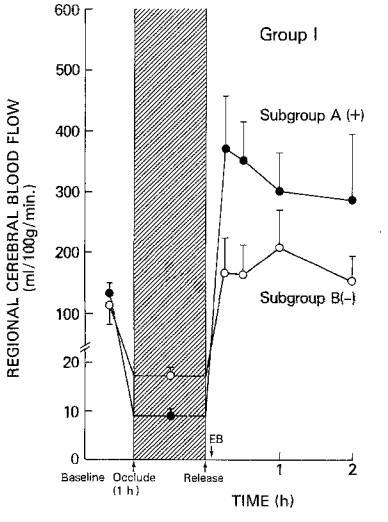


Fig. 2. Group I. Cats injected with EB shortly after release of occlusion and killed 2 h later

The leakage of EB, clearly related to ischemia, was characterized by distinct, diffuse blue discolorations of various intensities observed predominantly in the caudate and the cerebral cortex on the side of the MCA occlusion (Fig. 8). In the caudate, such uniformly diffuse blue discoloration was most frequent in the superior, lateral portions of the nucleus, whereas among the cortical regions within the MCA supply territory, extensive EB extravasations were most common in the ectosylvian cortex. Observations under the fluorescence microscope of such areas of blue discoloration of ischemic origin revealed spreading of the EB tracer through the vascular walls into the brain parenchyma with frequent, intense red-fluorescent staining of ischemically affected neurons.

Group I, on the basis of BBB behavior in the studied 13 sites on the side occlusion, could be subdivided into subgroup A (five sites) showing leakage

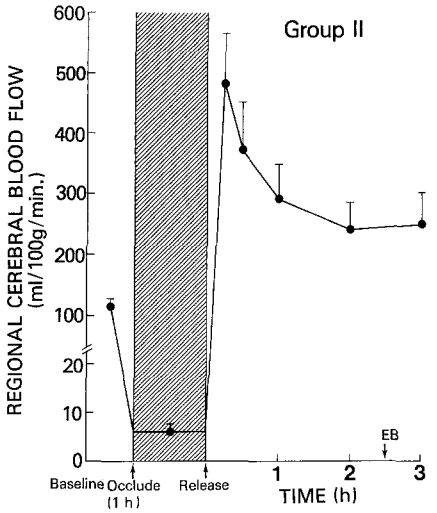


Fig. 3. Group II. Cats injected with EB 30 min before killing at 3 h after release of occlusion

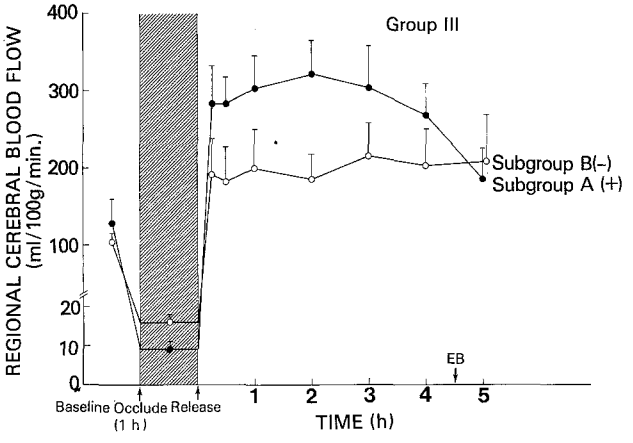


Fig. 4. Group III. Cats injected with EB 30 min before killing at 5 h

of EB into ischemic brain tissue, and subgroup B (eight sites) with no evidence of EB extravasation. Each subgroup was associated with different patterns of rCBF changes. Thus, during ischemic occlusion, the mean rCBF values in the EB-positive sites (subgroup A) were 8 ± 2 ml/100 g/min (Fig. 2, Table 2), whereas in EB-negative sites the mean rCBF was 17 ± 3 ml/100 g/min ($P < 0.05$). Following release of the occlusion, the sites in the EB-positive subgroup revealed a marked hyperemia, with the peak of rCBF 15 min after recirculation, whereas in EB-negative subgroup B, the reactive hyperemia was considerably reduced (Fig. 2, Table 2).

In group II, none of the 16 sites on the side of occlusion revealed evidence of EB leakage. In this group, during ischemia, the rCBF mean values on the occluded side were 6 ± 1 ml/100 g/min; the release of

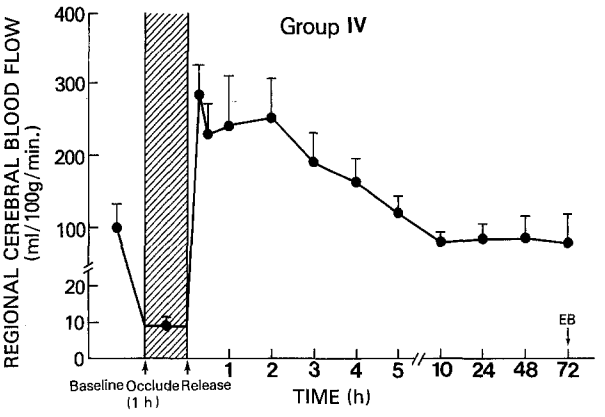


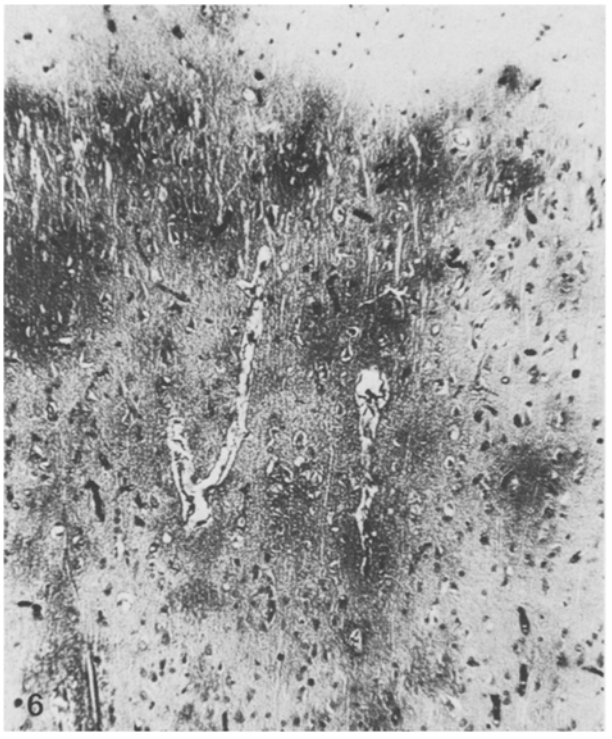
Fig. 5. Group IV. Cats injected with EB 30 min before killing at 72 h

the occlusion was followed by intense hyperemia, the highest rCBF values being recorded at 15 min after recirculation (Fig. 3, Table 2). The brains of five cats in group II, subjected to immunohistochemical demonstration of serum proteins, showed no evidence of EB extravasation. In all these brains, the sections processed according to the PAP procedure revealed areas of positive brown staining, specific for the presence of serum proteins, in the cortex or caudate or in both locations within the MCA blood supply territory on the occluded side. Microscopically, the PAP-positive staining was located diffusely in the neuropil or in the neurons, which otherwise revealed the signs of typical ischemic cell damage (Fig. 6).

Group III can be subdivided into subgroups A (eight sites) and B (seven sites) according to the presence or absence of EB extravasation in the regions associated with rCBF values below 30 ml/100 g/min during ischemia. In subgroup A, the sites with EB leakage revealed during MCA occlusion the rCBF values below 10 ml/100 g/min, followed by pronounced hyperemia after recirculation. On the other hand, the EB-negative regions (subgroup B) showed rCBF values during occlusion above 10 ml/100 g/min and considerably reduced hyperemia following recirculation (Fig. 4, Table 2).

In group IV, comprising cats killed 3 days after MCA occlusion, the six sites on the occluded side were associated with rCBF values during ischemia below 12 ml/100 g/min, and they showed marked hyperemia during the several hours of recirculation (Fig. 5, Table 2). All these sites revealed leakage of EB injected 30 min before killing.

The histological examination of the sections confirmed the location of the electrode tips within the cortical or caudate nucleus sites. In cats killed after 3 days, the electrode tips in the ischemic regions were seen to be surrounded by severely injured, partly necrotic brain tissue (Fig. 7).



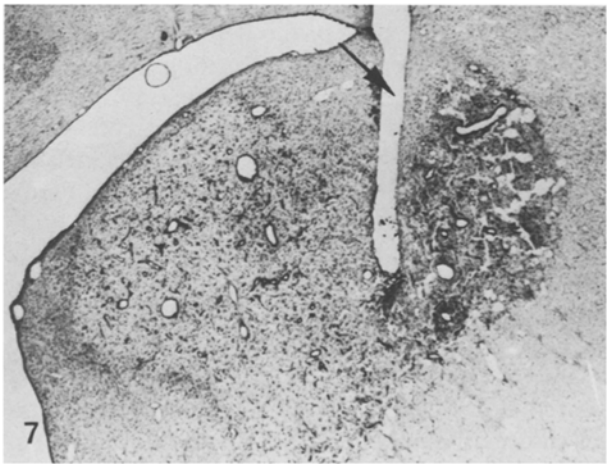


Fig. 6. Cerebral cortex on the side of the MCA occlusion of the cat killed 3 h after recirculation. Positive immunohistochemical staining for cat serum proteins is noticeable around blood vessels and in severely injured neurons. PAP method

Fig. 7. Cat killed after 72 h. The empty track in the caudate (*arrow*) corresponds to the platinum electrode, the recording from which during MCA occlusion revealed rCBF 8 ml/100 g/min. The brain parenchyma around the electrode shows a severe ischemic injury with ingrowth of new vascular channels into necrotic tissue filled with macrophages. Cresyl violet

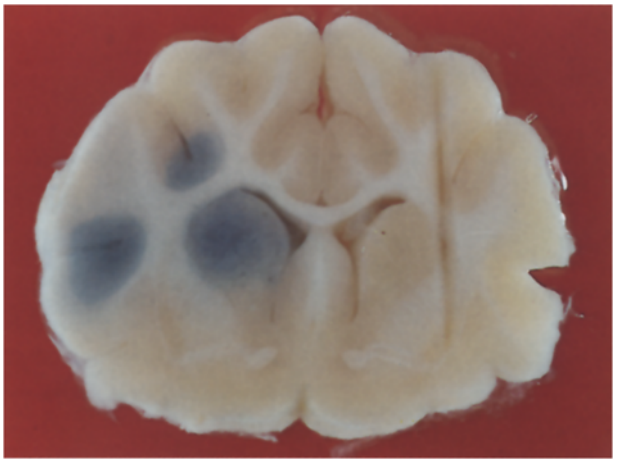


Fig. 8. Group III cat injected with EB 30 min before being sacrificed at 5 h after release of MCA occlusion. Diffuse blue discoloration in the nucleus caudatus and in the regions of the cerebral cortex supplied by the MCA

Discussion

The present observations demonstrate that following release of arterial occlusion, there can be two independent openings of the BBB to proteins, separated by a "refractory" period.

The first barrier opening, occurring promptly after recirculation, is associated with a pronounced reactive hyperemia, which almost invariably develops after release of arterial occlusion in areas subjected previously to a severe ischemia. In the present study, the first barrier opening was observed only in cats in which the reduction of rCBF during 1 h MCA occlusion, was below 15 ml/100 g/min and accompanied by a pronounced increase in rCBF from the base values following recirculation. This first barrier opening can be regarded as primarily "hemodynamic" in nature, induced by a drastic elevation of rCBF rushing through maximally dilated blood vessels resulting from acidosis and lack of autoregulation. The mechanism of this opening might be thus similar to that responsible for the BBB breakdown in acute systemic hypertension, which was shown to be related to the rate of increase in CBF and systemic blood pressure (Suzuki et al. 1984). Otherwise, Johansson and Nilsson (1977) reported that the BBB dysfunction in hypercapnic rats and those with epileptic seizures was related to the combined effects of high blood pressure and cerebral vasodilatation.

The "refractory" state of the barrier observed in animals injected with EB 30 min before killing at 3 h after recirculation (group II) is especially significant because it was demonstrable in animals which showed very severe ischemia (6 ± 1 ml/100 g/min) during the MCA occlusion, followed by a fourfold elevation of rCBF at 15 min after release of occlusion, and in which the first barrier opening was confirmed by the PAP staining. Otherwise, Hossmann and Olsson (1977) observed a refractory state of the BBB in cats in which attempts to produce EB extravasation by intravascular perfusion of the brain with mercury chloride, which easily breaks BBB under normal conditions, were unsuccessful in cats previously subjected to severe global ischemia, associated with electrophysiologic abolition of the pyramidal response.

There can be several explanations concerning mechanism of the "refractory" period, interposed between two barrier openings. A most likely explanation would be to assume that a progressively subsiding hyperemia and re-establishment of autoregulation remove conditions for the "hemodynamic" leakage, and thus a normal function of the barrier is re-established. Another explanation could be that the "refractory" period is related to inhibition of pinocytotic activity in the endothelium, which has been considered as a mechanism responsible for leakage of protein tracers observed in cerebral ischemia (Westergaard et al. 1976). Since the pinocytotic transport is regarded as an active, energy-dependent process, it is possible that postischemic disturbance in energy metabolism could temporarily inhibit the transport and leakage of proteins.

The mechanism for the second BBB opening remains obscure. This second opening after release of temporary occlusion seems to correspond to that occurring with characteristic delay in permanent arterial ligation, and it is invariably associated with severe ischemic tissue injury leading to infarction. It should be emphasized, however, that at the time of occurrence of delayed protein leakage, although the parenchymatous cellular elements may reveal an advanced, irreversible damage, the cerebral endothelium itself, including tight junctions, appears to be remarkably well preserved (Westergaard et al. 1976). This suggests that this barrier opening, not associated with any noticeable hemodynamic disturbance, is induced by some factors deriving from the severely damaged tissue.

A direct relationship between entry of serum proteins into extracellular spaces and increment in the water content was recently demonstrated in an experimental model in which the opening of the BBB to proteins was not associated with any evidence of brain injury (Kuroiwa et al. 1985). In cerebral ischemia, an opening of the barrier for proteins introduces an element of vasogenic brain edema, which greatly aggravates the existing cytotoxic edema, and it may adversely influence the microcirculation in the adjacent areas of the penumbra and lead to a peripheral expansion of the ischemic lesions (Klatzo 1985).

The parenchyma-generated factors responsible for barrier opening to proteins might be of a different nature. The release of substances, such as kinins, fatty acids, serotonin, and prostaglandins from severely damaged tissue which can produce edema and changes in vascular permeability has been mentioned in numerous studies (Baethmann 1978; Pappius and Wolfe 1984). It appears then that the search and the elucidation of tissue factors responsible for the barrier opening is imperative since the prevention of the protein extravasation in cerebral ischemia might have significant beneficial effect on the course of the ischemic lesion, and some recent experimental findings seem to support such an assumption (Wagner et al. 1983).

Acknowledgements. The excellent technical assistance of Mrs. I. Fenton and the editorial work on the manuscript of Mrs. H. Mittelman are greatly appreciated. We are indebted to Drs. J. D. Bacher and J. F. Harwell for providing excellent surgical facilities and to Mr. C. W. Casey and Ms. L. M. Nierzwicki for their helpful statistical assistance.

References

Aukland K, Bower BF, Berliner RW (1964) Measurement of local blood flow with hydrogen gas. Circ Res 164–187

Baethmann A (1978) Pathophysiological and pathochemical aspects of cerebral edema. Neurosurg Rev 1:85–96

Broman T (1949) The permeability of the cerebrospinal vessels in normal and pathological conditions. Munksgaard, Copenhagen

Cervos-Navarro J, Artigas J, Mrsulja BJ (1983) Morphofunctional aspects of the normal and pathological blood-brain-barrier. Acta Neuropathol [Suppl] (Berl) VIII:1–19

Fein JM, Willis J, Hamilton J, Parkhurst J (1975) Polarographical measurement of local cerebral blood flow in the conscious and anesthetized primate. Stroke 6:42–51

Fujimoto T, Walker JT, Jr, Spatz M, Klatzo I (1977) Pathophysiologic aspects of ischemic edema. In: Pappius HM, Feindel W (eds) Dynamics of brain edema. Springer, Berlin Heidelberg New York, pp 171–192

Haining JL, Turner MD, Pantall RM (1968) Measurement of local cerebral blood flow in the unanesthetized rat using hydrogen clearance method. Circ Res 23:313–324

Halsey JH, Capra NF, McFarland RS (1977) Use of hydrogen for measurement of regional cerebral blood flow-problem of intercompartmental diffusion. Stroke 8:351–357

Hossmann K-A, Olsson Y (1972) Functional aspects of abnormal protein passage across the blood-brain barrier. In: Reulen HJ, Schurman K (eds) Steroids and brain edema. Springer, Berlin Heidelberg New York, pp 9–17

Ito U, Spatz M, Walker JT, Jr, Klatzo I (1975) Experimental cerebral ischemia in Mongolian gerbils. I. Light-microscopic observations. Acta Neuropathol (Berl) 32:209–223

Johansson B, Nilsson B (1977) The pathophysiology of the blood-brain barrier dysfunction induced by severe hypercapnia and by epileptic brain activity. Acta Neuropathol (Berl) 38:153–158

Juhler M, Barry DI, Offner H, Konat G, Klinken L, Paulson OB (1984) Blood-brain and blood spinal cord barrier permeability during the course of experimental allergic encephalomyelitis in the rat. Brain Res 302:347–355

Klatzo I (1975) Pathophysiologic aspects of cerebral ischemia. In: Tower DB (ed) The nervous system. The basic neurosciences, vol 1. Raven Press, New York, pp 313–322

Klatzo I (1983) Disturbance of the blood-brain barrier in cerebrovascular disorders. Acta Neuropathol [Suppl] (Berl) VIII:81–88

Klatzo I (1985) Brain oedema following brain ischemia and the influence of therapy. Br J Anaesth 57:18–22

Kuroiwa T, Cahn R, Juhler M, Goping G, Campbell G, Klatzo I (1985) Role of extracellular proteins in the dynamics of vasogenic brain edema. Acta Neuropathol (Berl) 66:3–11

Nagy Z, Huttner I (1983) Opening of tight junctions in cerebral endothelium by hyperosmolar mannitol. Acta Neuropathol [Suppl] (Berl) VIII:143

Nishimoto K, Wolman M, Spatz M, Klatzo I (1978) Pathophysiologic correlations in the blood-brain barrier damage due to air embolism. In: Cervos-Navarro E, Betz E, Ebhardt G, Ferszt R, Wullenweber R (eds) Pathology of cerebral microcirculation. Adv Neurol, vol 20. Raven Press, New York, pp 237–244

Olsson Y, Crowell RM, Klatzo I (1971) The blood-brain barrier to protein tracers in focal cerebral ischemia and infarction caused by occlusion of the middle cerebral artery. Acta Neuropathol (Berl) 18:89–102

Pappius H, Wolfe L (1984) Effect of drugs on local cerebral glucose utilization in traumatized brain: Mechanisms of action of steroids revisited. In: Go KG, Baethmann A (eds) Recent progress in the study and therapy of brain edema. Plenum Press, New York, pp 11–26

Pasztor E, Symon L, Dorsch NWC, Branston N (1973) The hydrogen clearance method in assessment of blood flow in cortex, white matter an deep nuclei of baboons. Stroke 4:556–566

Petito CK, Pulsinelli WA, Jacobson G, Plum F (1982) Edema and vascular permeability in cerebral ischemia: Comparison between ischemic neuronal damage and infarction. J Neuropathol Exp Neurol 41:423–436

Romano AG, Steinmetz JE, Patterson MM (1980) A versatile cat-restraint system. Behav Res 12:455–458

Senter HJ, Burgess DH, Metzler J (1978) An improved technique for measurement of spinal cord blood flow. Brain Res 149:197–203

Suzuki R, Yamaguchi T, Kirino T, Orzi F, Klatzo I (1983) The effects of 5-min ischemia in Mongolian gerbils: I. Blood-brain barrier, cerebral blood flow, and local cerebral glucose utilization changes. Acta Neuropathol (Berl) 60:207–216

Suzuki R, Nitsch C, Fujiwara K, Klatzo I (1984) Regional changes in cerebral blood flow and blood-brain barrier permeability during epileptiform seizures and in acute hypertension in rabbits. J Cereb Blood Flow Metab 4:96–102

Wagner H, Cahn R, Kuroiwa T, Ting P, Yamaguchi T, Klatzo I (1983) Role of the blood-brain barrier opening to proteins in pathophysiology of cerebral ischemia. J Cereb Blood Flow Metab [Suppl 1] 3:S417–S418

Westergaard E, Go KG, Klatzo I, Spatz M (1976) Increased permeability of cerebral vessels to horseradish peroxidase induced by ischemia in Mongolian gerbils. Acta Neuropathol (Berl) 35:307–325

Young W (1980) H_2 clearance measurement of blood flow: A review of technique and polarographic principles. Stroke 11:552–564

Received February 13, 1985/Accepted May 13, 1985