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Blood-Brain Barrier Impairment by Low pH Buffer Perfusion via the Internal Carotid Artery in Rat*

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Summary. Krebs-Ringer bicarbonate buffer (KRB) adjusted by lactic acid to low pH levels (6.6, 6.2, 6.0, 5.8, 5.5) was perfused via the internal carotid artery as a bolus into rat brain hemispheres. In one group of animals, the fluid phase tracer horseradish peroxidase (HRP) was perfused immediately following the low pH treatment. In the other group of animals, the brain hemispheres were fixed and the endothelial cells were stained with colloidal iron (CI) at pH 1.8.

Widespread extravasation of HRP was detected indicating blood-brain barrier (BBB) opening to this tracer in hemispheres perfused with KRB at pH 6.2, 6.0, 5.8, or 5.5. HRP was seen in pools of endothelial tight junctions. Endothelial cell injury reflected by swelling and influx of HRP into the cytoplasm was occasionally encountered. CI evenly decorated the negatively charged surface of endothelial cells in the control brains, in contrast to markedly diminished iron binding capacity of endothelial cells in low pH-treated hemispheres.

Our results suggest that the ionic milieu influences the negatively charged cell surface sialoglycoproteins and glycolipids, which are integral parts of the BBB system.

Key words: Cerebral endothelium – Endothelial surface coat – Low pH buffer perfusion – Horseradish peroxidase – Colloidal iron staining at pH 1.8.

Introduction

Lactic acidosis, which accompanies a local fall in brain pH is well documented in experimental ischemia of brain tissue (Ljunggren et al. 1974; Mabe et al. 1983; Nemoto and Severinghaus 1974; Nemoto and Frinak 1981). Substantial evidence indicates that brain acidosis plays an important role in the pathogenesis of ischemic brain edema. The severity of brain edema appears to correlate with the degree of tissue acidosis (Takahashi 1966). The transport function of cerebral microvascular endothelium is also pH-dependent (Oldendorf 1971; Oldendorf et al. 1979). Fall in brain pH resulting from local disturbance of microcirculation have been implicated as a factor of endothelial cell injury (Kalimo et al. 1981; Paljärvi et al. 1983).

The luminal surface of cerebrovascular endothelial cells represents the true interface between circulating blood and brain tissue. The surface coat of endothelial cells is negatively charged. Distribution and movement of the surface charge influence various membrane-associated processes and may affect the barrier function of this cell layer (Nagy et al. 1983; Skutelsky et al. 1975). Blood-brain barrier (BBB) opening to horseradish peroxidase (HRP) was demonstrated following perfusion of the polycation protamine sulfate via the internal carotid artery. The free negative sites of endothelial cell surface react rapidly with this polycation resulting in cell injury and opening of paracellular avenues between endothelial cells (Nagy et al. 1983). On the basis of these observations it is reasonable to suggest that the ionic milieu of the cerebral microvasculare may affect the surface charge and barrier function of cerebral endothelium. In the present study we investigated the effect of a Krebs-Ringer bicarbonate buffer (KRB) set at a low pH with lactic acid on the BBB within a cerebral hemisphere of rats perfused with this solution.

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Materials and Methods

Experiments were carried out on male Wistar rats weighing 300–350 g. The animals were anesthetized i.p. with Nembutal. The right common carotid artery was catheterized, and the ex-

Table 1. Experimental Group Data

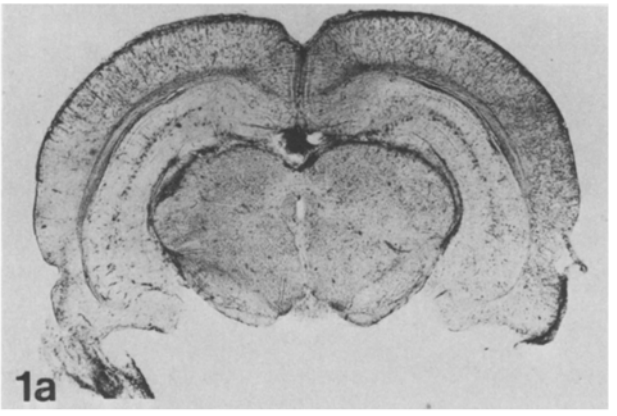
Intracarotid perfusion with	No. of animals	
	in experiment with HRP	in experiment with CI staining
KRB at pH 7.4 (control)	2	2
KRB at pH 6.6	2	2
6.2	2	2
6.0	3	3
5.8	4	3
5.5	4	2

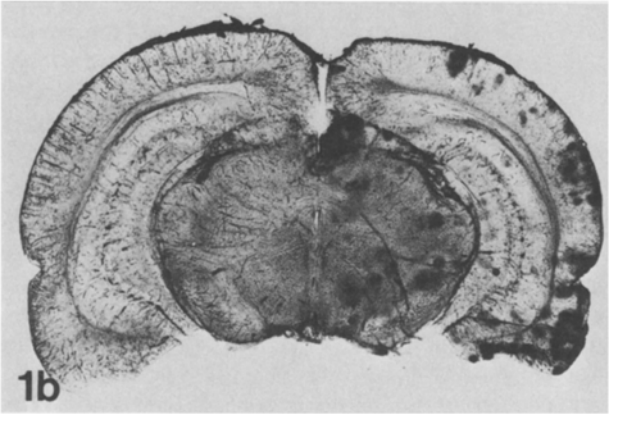
KRB, Krebs-Ringer bicarbonate buffer; HRP, horseradish peroxidase; CI, colloidal iron staining at pH 1.8

ternal branch was ligated for selective perfusion of the ipsilateral hemisphere, as we described in detail previously (Nagy et al. 1979). Perfusion was initiated for 20 s using KRB buffer at pH 7.4 as a bolus to wash out the blood from the vascular bed. A perfusion rate of 5 ml/min was maintained while opening the ipsilateral jugular vein. This perfusion rate results in about 100–120 mmHg level of intravascular pressure (Nagy et al. 1979). Low pH buffer applied for 90 s subsequently were used immediately thereafter with the same perfusion rate to avoid blood recirculation into the perfused hemisphere. In the experimental group the pH level of KRB buffer was adjusted by lactic acid to 6.6, 6.2, 6.0, 5.8, or 5.5, respectively. In control animals KRB buffer at pH 7.4 was infused for 90 s with the same perfusion rate as in the experimental animals (Table 1).

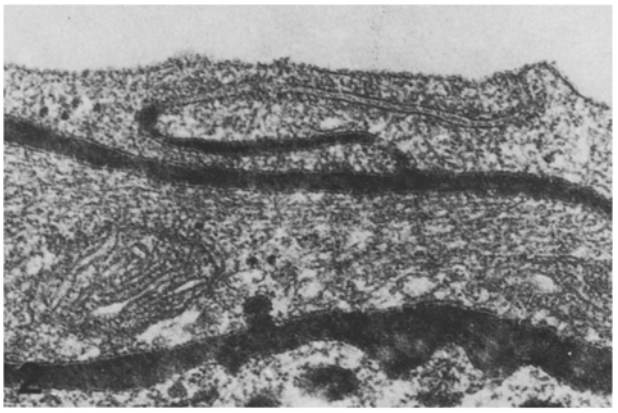
In part of each animal group (Table 1), HRP, Sigma type II, was perfused in a concentration of 20 mg/ml in KRB buffer at pH 7.4 via the internal carotid artery for 1 min with a perfusion rate of 5 ml/min. This was followed by perfusion of the brain, with modified Karnovsky's fixative for 20 min (Nagy et al. 1979), applying the same perfusion rate as used for the experimental procedures. The fixative contained 1% freshly prepared paraformaldehyde and 1.25% purified glutaraldehyde in 0.1 N sodium cacodylate buffer (pH 7.4) with 5% sucrose. The perfusion was continued with a more concentrated Karnovsky's fixative, containing 2% paraformaldehyde and 2.5% glutaraldehyde with the same buffer for another 20 min. After removal, the brains were stored in the fixative overnight at 4° C and washed for 2 h in 0.1 N sodium cacodylate buffer. Histochemical reaction for demonstration of HRP was performed in 50 μm tissue slices prepared by a tissue chopper. The slices were incubated for 1 h at room temperature in Graham-Karnovsky's medium as described previously (Nagy et al. 1979). The tissue slices were then postfixed for 90 min with 1% OsO_4 in veronal-acetate buffer at pH 7.4, followed by washing with the same buffer, and treated for 2 h with 2% uranyl acetate at pH 5.2. After these steps the tissue were dehydrated in graded ethanols and embedded in Araldite. In addition, 1 mm-thick coronal brain slices incubated for demonstration of HRP reaction product were cleared with xylol and mounted for macroscopic evaluation.

In other animals from each group, colloidal iron (CI) staining at pH 1.8 was performed on brain tissues fixed immediately after perfusion of the KRB at the various pH levels specified above. Fixation of the treated hemisphere was carried out by perfusion of modified Karnovsky's fixatives through the cannulated carotid artery. The brains were then removed carefully and stored in fixative overnight at 4° C. Sections, 50 μm





Figs. 1a–c. One-millimeter-thick coronal section of rat brains perfused through the right common carotid artery for 90 s with **a** Krebs-Ringer bicarbonate buffer solution (KRB), pH 7.4 (control); **b** KRB adjusted by lactic acid to pH 6.2; **c** KRB adjusted by lactic acid to pH 5.5. The right hemispheres were perfused subsequently with 100 mg horseradish peroxidase (HRP) in 5 ml KRB for 1 min. In the control brain, HRP labels only occasional vascular segments in the perfused hemisphere. In hemisphere perfused with KRB at pH 6.2, HRP reaction product labels numerous vascular segments and extends into the neuropil in a patchy fashion In hemisphere perfused with KRB at pH 5.5, the tracer diffusely labels cortical and subcortical regions



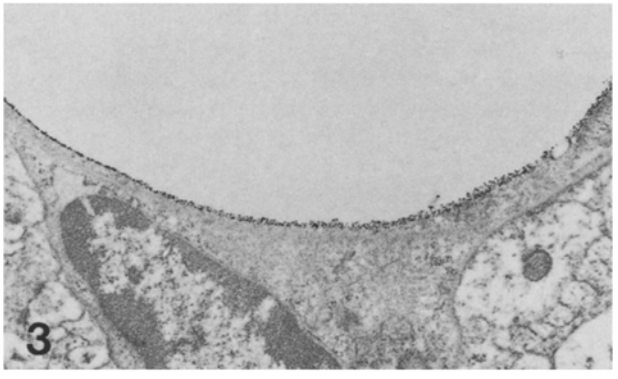


Fig. 2. Electron micrograph of small artery segment from rat brain hemisphere perfused with KRB at pH 5.8 followed by HRP. Electron-dense reaction product of HRP is evident in compartments of tight junction and in subendothelial space; ×43,560

Fig. 3. Colloidal iron (CI) staining, pH 1.8, in control hemisphere. Iron particles evenly decorate luminal surface of capillary endothelial cell; ×40,300

Fig. 4. CI staining, pH 1.8, in hemisphere perfused with KRB at pH 5.8. Iron staining of endothelial cell surface is diminished. Note marked edema of endothelial cell; ×42,570

thick, were rinsed in 20% acetic acid (pH 1.8) and placed for 3 h in a positively charged CI solution prepared according to the method of Rinehart and Abul-Haj (1951). After treatment with CI, the sections were postfixed for 2 h with 1% OsO_4 in veronal-acetate buffer at pH 7.4. The tissue slices were dehydrated and embedded in Araldite.

One micrometer unstained sections were prepared from all Araldite-embedded specimens for selecting areas for electron microscopy.

Results

In control brain hemispheres perfused with KRB buffer at pH 7.4, HRP labeled only occasional vascular segments (Fig. 1 a).

In experimental brain hemispheres perfused with low pH buffer, tracer extravasation was evident at pH levels of 6.2, 6.0, 5.8, and 5.5. While perfusion of the buffer at pH 6.2 induced only focal impairment of the BBB, perfusion of the buffer at very low pH (5.8 or 5.5) resulted in extensive extravasation of HRP in the affected henispheres (Figs. 1 b, c). Electron microscopy of hemispheres treated with low pH buffer perfusion revealed electron-dense reaction product in the subendothelial spaces and in the intercellular compartments of the surrounding neuropil. HRP tracer labeled pools of interendothelial tight junctions in occasional vascular segments (Fig. 2). Labeled plasmalemmal vesicles were only rarely encountered.

CI at pH 1.8 evenly decorated the luminal surface of the endothelial cells in the brains of control animals (Fig. 3). In contrast, the iron-binding capacity of endothelial cell surface markedly diminished as little or no iron staining could be observed following low pH buffer perfusion (Fig. 4). Edema of individual endothelial cells was also noted in the low pH buffer-perfused group (Fig. 4).

Discussion

Hemispherial perfusion of KRB buffer adusted to a low pH with lactic acid probably resulted in a rapid fall of pH within the cerbrovascular bed in our short-term rat brain perfusion model. The extravation of HRP, visualized by light and electron microscopy, indicated that low pH buffer perfusion induced an alteration of the BBB to the tracer in this model. This low pH-related BBB opening was not reversed by subsequent perfusion of the tracer and the fixatives at pH 7.4. The diminished colloidal iron binding capacity of the affected vascular segments reflected alteration of the negatively charged endothelial surface coat. The BBB impairment specified here is similar to that found previously following polycation perfusion into rat hemispheres (Nagy et al. 1981, 1983).

The cell surface glycoproteins and glycolipids containing terminal sialic acid constituents as well as carboxylic groups of proteins have the staining characteristics of polyanions. Sulfated groups of glycoproteins and glycosaminoglycans are the other major source of negative electric charge in cell surface

(Simionescu et al. 1981). The anionic elements on the endothelial surface coat are probably not affected by a small change in blood pH. The pK of sialic acid is around 2.6, which means that a large percentage of these anionic groups are ionized and probably negatively charged even at very low systemic blood pH (Fraser et al. 1978). In local circulatory disturbances, however, tissue pH may drop to a level effecting the anionic surface charge of endothelium. A pH-dependent reversible aggregation of integral membrane proteins in human erythrocyte ghosts has been demonstrated by freeze fracture electron microscopy (Pinto da Silva 1972). At pH level in the vicinity of 5.5, particle aggregation occurred within 2–4 min. Clusters of anionic sites of red blood cell membrane were visualized similarly by CI following incubation at pH 5.5 (Nicolson 1973). Integral membrane proteins of cell membranes are anchored partly by their negatively charged sugar residues to the cell surface. Low pH or polycationic agents (Nagy et al. 1983) may affect this "electrostatic anchor" resulting in particle aggregation (Pinto da Silva 1972) followed by internalization or detachment of anionic groups (Skutelsky and Danon 1976) resulting in decreased colloidal iron-binding capacity of the cell surface. How these membrane phenomena influence the structural integrity of lateral endothelial cell membrane and the barrier function of tight junctions, however, are unknown.

Incomplete ischemia in the cases of impaired blood circulation represents a high risk of brain lactic acidosis and of fall in brain pH. Recirculation replenishes the brain rapidly with glucose. Glucose is quickly converted to lactate by activated glycolytic enzymes causing a further fall in brain pH (Ljunggren et al. 1974). As stated in the introductory remarks, anaerobic lactate production in the development of ischemic brain injury is a basic mechanism of ischemic encephalopathy (Kalimo et al. 1981; Ljunggren et al. 1974; Mabe et al. 1983; Nemeto and Frinak 1981). This mechanism is operative if ischemia is combined with an excessive increase of tissue lactate concentrations and results in a pH fall to levels around 6.0 or below (Paljärvi et al. 1983). The low pH of the ischemic brain is one of the factor leading to BBB impairment in ischemic brain edema.

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