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Short Report

Differential expression of an endothelial barrier antigen between the CNS and the PNS

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ABSTRACT

A monoclonal antibody to an antigen (EBA) expressed by neural endothelial cells (EC) was used to investigate any difference in the distribution of EBA between the CNS and PNS. Pre-embedding ultrastructural cytochemistry of rat sciatic and optic nerves was undertaken using anti-EBA, detected with a silver-enhanced gold-conjugated secondary antibody. LM immunocytochemical localisation of EBA was also performed using an HRP-conjugated secondary antibody. EC of pial and parenchymal optic nerve vessels were strongly immunopositive for EBA. Vessels of the dura were negative. At the EM level EBA was observed on the EC luminal surface. In contrast, EC of sciatic nerve were either negative or only weakly immunopositive. The molecular characteristics and function of EBA are largely unknown. Therefore the functional significance of the present findings remains to be determined.

Key words: Blood–brain barrier; blood–nerve barrier.

INTRODUCTION

A mouse monoclonal antibody (IgG1) raised by immunisation with an homogenate of rat brain has been shown to react with endothelial cells (EC) in the rat nervous system (Sternberger & Sternberger, 1987). Since the antibody binding was evident in EC with highly selective barrier properties, the antibody was named anti-endothelial-barrier antigen (anti-EBA). In CNS regions lacking a blood–brain barrier (BBB), where fenestrated vessels are present, as in the area postrema, microvessels were only weakly reactive or nonreactive. The antibody did not react with neurons or glial cells. It was absent from EC at embryonic d 18 (Rosenstein et al. 1992) but present as early as 3 d post partum (Sternberger & Sternberger, 1987). Blood vessels in other organs, including heart, muscle, intestine and liver, were negative for anti-EBA (Sternberger & Sternberger, 1987). Following stab wound injury to the rat brain, EC of affected microvessels failed to show anti-EBA reactivity concurrent with a loss of BBB properties, with reactivity returning to normal by 3–4 wk (Rosenstein et al. 1992). Similarly transplants of fetal cortex into adult rat brain lacked EBA expression in EC at 2 wk, but were immunopositive by 4 wk (Rosenstein et al. 1992). In lesions of experimental allergic encephalomyelitis (EAE) microvessels surrounded by inflammatory cells lacked anti-EBA reactivity which was re-expressed as the lesions resolved (Sternberger et al. 1989). In aged rats EBA-positive microvessels were significantly reduced in number in the hippocampus but not in other cerebral cortical areas (Mooradian et al. 1993).

The molecular composition and structure of EBA are little understood while its function is unknown. It consists of a protein triplet of 23.5, 25 and 30 kDa as shown by immunoblots prepared from rat brain microvessels (Sternberger & Sternberger, 1987). The only indication of its possible function is derived from its restricted morphological distribution (Sternberger & Sternberger, 1987). To clarify its distribution

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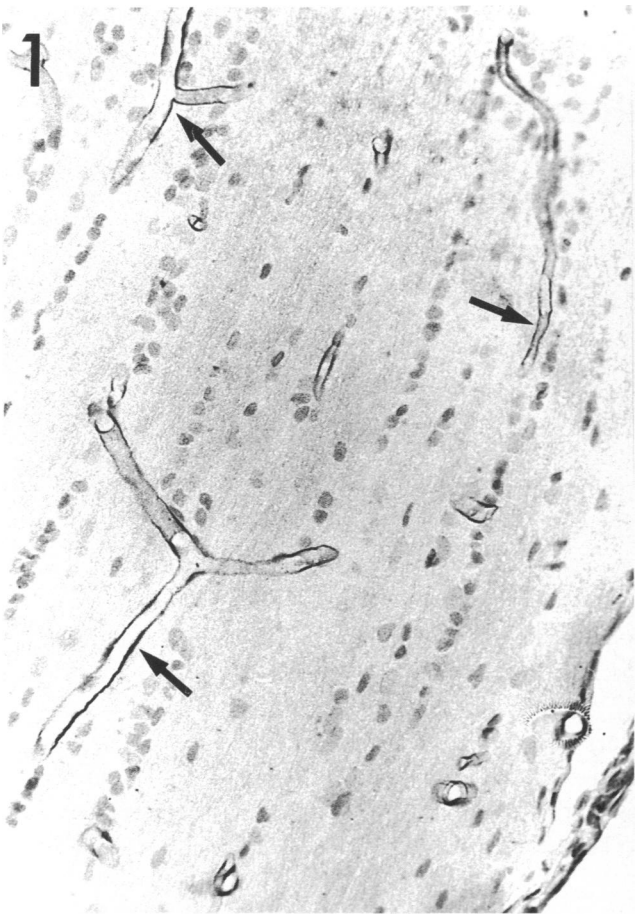

Fig. 1. Longitudinal section of optic nerve showing parenchymal microvessels which are immunopositive for EBA (arrows). Lightly counterstained with haematoxylin. ×430.

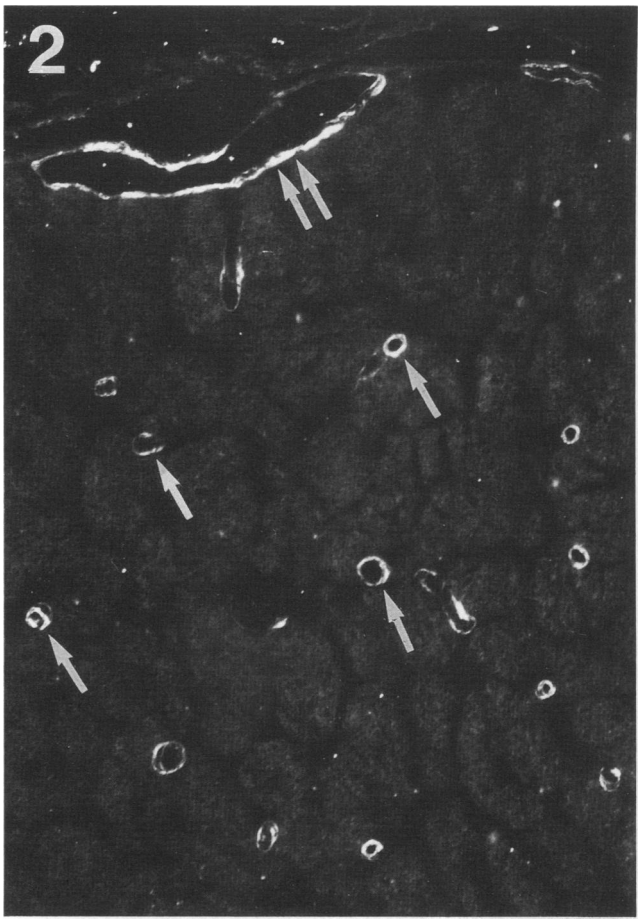

Fig. 2. Transverse section of optic nerve in dark field illumination. Both parenchymal (single arrows) and pial (double arrows) vessels are strongly EBA-positive. ×340.

further, its presence in the CNS and PNS was compared. An ultrastructural study was undertaken employing the silver-enhanced gold-conjugated antibody technique.

MATERIALS AND METHODS

Twelve Sprague–Dawley male rats (200–300 g) were anaesthetised with i.m. Hypnorm (0.16 mg/kg body weight) following sedation with i.p. diazepam (0.2 mg/kg body weight). Vascular perfusion was initially with Dulbecco's phosphate-buffered saline (DPBS: pH 7.3, 320 mOsm at 37 °C) supplemented with minimum essential medium and gassed with 95% O_2:5% CO_2. This was followed with phosphate-buffered 0.2% glutaraldehyde–2% paraformaldehyde fixative (pH 7.3 at 4 °C) for 10 min. Sciatic nerves, optic nerves and eyes were removed and immersed in the same fixative for 2 h.

For ultrastructural cytochemistry, Vibratome sections (200 μm) of optic and sciatic nerves were incubated in the primary antibody (Sternberger & Sternberger, 1987): anti-EBA (mouse monoclonal antirat, diluted 1:100 in 50 mM-TBS, pH 7.6, containing 1% normal goat serum, overnight; obtained from Affiniti Research Products Ltd, Nottingham, UK). The sections were washed in TBS and incubated in the secondary antibody (1 nm gold-conjugated goat antimouse IgG, diluted 1:50 in 20 mM-TBS, pH 7.0 for 3 h; obtained from Affiniti). As controls the primary antibody was omitted. After washing in

Fig. 3. Immunogold labelling of EC for EBA (silver-enhanced). (*A*) Parenchymal vessel from optic nerve showing heavy labelling with gold particles (arrows) on the luminal plasmalemma. ×45000. (*B*) Endoneurial vessel from sciatic nerve showing sparse labelling (arrows) of the luminal plasmalemma. ×22500. The variation in size and shape of the particles is a feature of the silver enhancement technique and has no significance in terms of EBA reactivity. EC, endothelial cells; ECN, endothelial cell nucleus; En, endoneurium; M, myelin; N, neuropil; SC, Schwann cell.

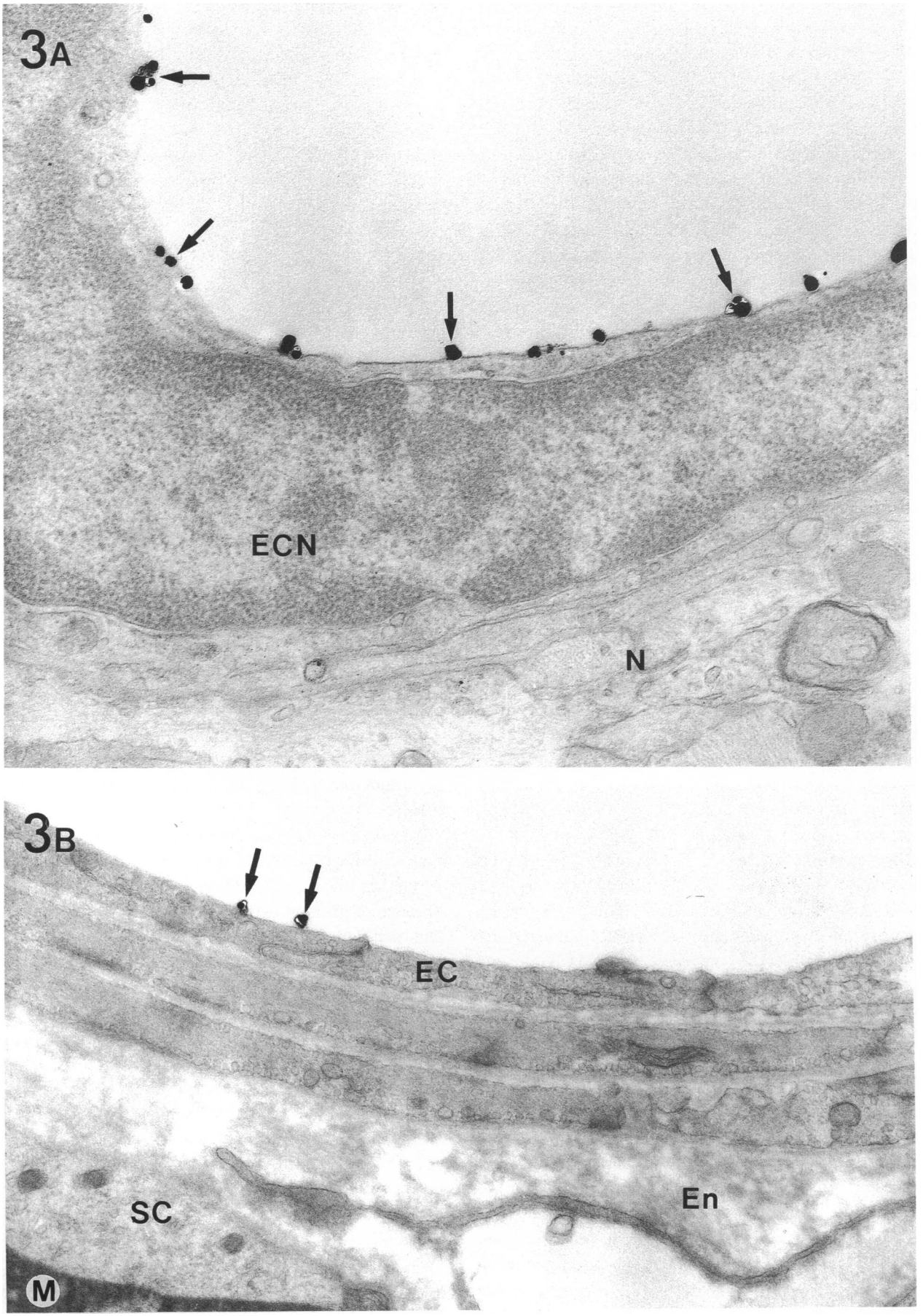

Fig. 3. For legend see opposite.

buffer, the gold-antibody conjugate was silver enhanced using a BioCell kit (Cardiff, UK) for 10 min. After further washing, the sections were osmicated (1% OsO_4 solution for 60 min), dehydrated (graded ethanols) and embedded in epoxy resin (EM laboratories Ltd, Reading, UK). Conventional ultrathin sections were examined by electron microscopy.

For light microscopical cytochemistry, segments of the sciatic nerve and the posterior part of the eye were processed conventionally for paraffin wax embedding. The sciatic nerve, optic nerve head and intraorbital optic nerve were examined. Sections (8–10 μm) were collected on glass slides precoated with poly-L-lysine, dewaxed with xylene and transferred to absolute alcohol. Sections were incubated for 10 min in normal goat serum 1:10 in TBS. Endogenous peroxidase was blocked with 0.5% hydrogen peroxide in absolute methanol for 30 min (sections of spleen, both blocked and unblocked, were used as controls). After rinses in distilled water and TBS, sections were incubated in anti-EBA (diluted 1:500 and 1:1000 in 50 mM-TBS, pH 7.6, overnight; Affiniti). Sections were incubated after TBS rinses, with goat-antimouse horseradish peroxidase (HRP) conjugate (1:100 in 20 mM-TBS pH 7.0 for 3 h; obtained from Dako, High Wycombe, UK). After TBS rinses, sections were exposed to 0.1% diaminobenzidine tetrahydrochloride (DAB) with 0.02% hydrogen peroxide for 2–5 min followed by rinses in distilled and tap water. Sections were stained with haematoxylin and conventionally mounted for examination by light microscopy (LM).

RESULTS

Optic nerve, examined by LM (Figs 1,2), showed immunopositive vessels in the parenchyma and pia while vessels in the dura were negative. Capillaries were more strongly labelled with HRP than arterioles and venules. Electron microscopy (EM) confirmed that labelling was confined to pial and parenchymal vessels. Gold particles were numerous along the luminal membrane of the EC in tissue treated with the gold-antibody conjugate (Fig. 3*A*). Although the nature of the pre-embedding labelling technique limited antibody access to the EC cytoplasm and abluminal surface, there was no evidence of labelling in these areas in EC at the Vibratome faces which had unrestricted exposure to antibodies. Controls were negative.

Sciatic nerve examined by LM (paraffin wax sections) showed no labelling in epineurial, perineurial or endoneurial vessels. By using polyester wax as the embedding medium and a long exposure (10 min) to DAB, weak immunoreactivity was obtained but in only a minority of endoneurial vessels. By EM epineurial and perineurial vessels were seen to be unlabelled. Endoneurial vessels were also mostly EBA-negative, but in a minority of vessels there was sparse labelling (Fig. 3*B*) though even here large segments of the luminal surface were free of gold labelling. The control tissues were unreactive for EBA.

DISCUSSION

This study demonstrates a heterogeneity in EC within the nervous system, even between vessels showing barrier properties. It has previously been shown that EBA immunoreactivity is absent or reduced from fenestrated vessels which occur in normal CNS regions lacking a BBB (Sternberger & Sternberger, 1987), from microvessels in EAE lesions (Sternberger et al. 1989) and from some microvessels in the aged hippocampus (Mooradian et al. 1993). We have shown in this study by both LM and EM immunocytochemistry that parenchymal vessels in the optic nerve are highly reactive for EBA while endoneurial vessels of the sciatic nerve are unreactive or only slightly reactive. This appears not to have been demonstrated previously and contrasts with the observation of Sternberger & Sternberger (1987) that EC in the PNS expressed EBA. These workers did not state the PNS site examined and it is possible that a degree of heterogeneity exists within the PNS. This may relate to differences between nerves or to proximity to the CNS.

Astrocytes play an important, though still ill-defined, role both in inducing and maintaining BBB properties in EC (Bradbury, 1993). However, the absence of an astrocytic, or as yet unknown equivalent, cellular role in maintaining blood–nerve barrier function is unlikely to be a complete explanation for the difference in EBA labelling, since EBA is absent from EC in some regions of the normal and pathological CNS in both of which astrocytes are present (Sternberger & Sternberger, 1987, 1989; Rosenstein et al. 1992). In addition EBA reactivity has also been described in some cells in the skin, tentatively identified as Langerhans cells (Sternberger & Sternberger, 1987), which are known to participate in the presentation of antigen in association with major histocompatibility complex (MHC) class II molecules (Sternberger & Sternberger, 1987). However, it is unlikely that anti-EBA recognises MHC class II antigens, which are not expressed on EC of the normal CNS (Welsh et al. 1993). Furthermore the

evidence for EC of the normal CNS acting as antigen presenting cells is now considered insubstantial (Male, 1992). Antibody binding to transferrin receptor has been demonstrated in brain EC and it has been proposed that EBA might be a cell surface receptor (Sternberger et al. 1989). However, the absence of EBA from most EC outside the nervous system, together with its presence on Langerhans cells, makes the transport receptor hypothesis difficult to interpret.

Anti-EBA is one of 5 monoclonal (mAb) antibodies (reviewed by Dermietzel & Krause, 1991) which show BBB specificity. Anti-EBA is apparently distinct from 3 of these which also bind to EC at the BBB while the fourth is immunoreactive to an antigen of the pericyte plasmalemma. An mAb, termed HT7, with an MW of 74 kDa, has been shown to react specifically with chick EC at the BBB plus a plasmalemmal antigen of embryonic blood cells. A mouse mAb, 1W5, which recognises neurothelin (A 43 kDa glycoprotein) at the BBB has also been described. Neurothelin is present not only on EC but also on other neural tissue and some neuroepithelial derivatives. A further mAb appears to be distinct. It labels EC cytoplasm, luminal membranes and an extracellular layer at the rat BBB but its molecular characteristics are not known. Anti-EBA is exceptional in that other BBB-specific monoclonal (and polyclonal) antibodies also react with at least one of the class of transporting epithelia (Dermietzel & Krause, 1991). The functional significance of this difference remains to be elucidated.

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