Endothelial glycoconjugates: a comparative lectin study of the brain, retina and myocardium

J. G. LAWRENSON, J. P. CASSELLA, A. J. HAYES, J. A. FIRTH AND G. ALLT

1 Reta Lila Weston Institute of Neurological Studies, Windesyer Building, University College London, 2 Applied Vision Research Centre, Department of Optometry and Visual Science, City University, and 3 Biomedical Sciences Division, Imperial College School of Medicine, London, UK

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ABSTRACT

There is evidence that the endothelial cell (EC) glycocalyx is a significant determinant of vascular permeability, acting as a charge-size filter to permeant molecules. We have therefore examined its oligosaccharide composition in 3 classes of microvessel with differing permeabilities. EC in rat brain, retina and myocardium were labelled with a panel of lectins and subjected to a semiquantitative analysis. Surprisingly, no substantial differences were evident for any lectin labelling between the 3 microvessel types despite their marked morphophysiological diversity. In particular, all showed substantial sialic acid expression, with *Maackia amurensis* (MAA) labelling sialic acid in an \( \alpha_2–3 \) linkage to \( \beta \)-galactose and *Sambucus nigra* (SNA) recognising sialic acid in an \( \alpha_2–6 \) linkage to \( \beta \)-galactose. *Arachis hypogaea* (PNA) binding after neuraminidase digestion indicated the presence of Gal \( \beta_1–3 \)Gal\( \alpha \)-Nac attached to terminal sialic acid. The results therefore show that the sequences NeuNAc \( \alpha_2–3 \)Gal \( \beta_1–3 \)Gal\( \alpha \)-Nac and NeuNAc \( \alpha_2–6 \)Gal \( \beta_1–3 \)Gal\( \alpha \)-Nac are strongly expressed in the 3 microvessel types irrespective of their permeability properties. This homogeneity suggests that these lectin ligands may be involved in a common set of EC functions, e.g. cell:cell and cell:matrix interactions. However, we cannot rule out the possibility that glycocalyx differences may exist between vessels in the paracellular cleft which may alter its filtration properties.

Key words: Lectins; blood-brain barrier; blood-retinal barrier; myocardial endothelial cells.

INTRODUCTION

Under normal conditions continuous capillaries in different tissues show marked variability in their passive permeability or ‘leakiness’. The precise mechanisms for this nonselective transport of large and small hydrophilic molecules across the endothelial cell (EC) wall are still the subject of some debate (Rippe & Haraldsson, 1994; Michel, 1996). Two possible routes have been proposed: vesicular (caveolar) transport and a pathway via the paracellular cleft. There has been a long-standing controversy regarding the existence of a vesicular transport system, although recent evidence substantiates its role, particularly as a ‘large-pore’ pathway during raised permeability states, in noncerebral capillaries (Rippe & Haraldsson, 1994; Michel, 1996). By contrast, it is widely recognised that an important molecular-restrictive element is the paracellular cleft and it is clear that differences in junctional organisation between capillaries significantly alters the rate of nonspecific transport (Rippe & Haraldsson, 1994).

There is accumulating evidence that the endothelial glycocalyx is also a significant determinant of vascular permeability (Curry & Michel, 1980; Simionescu & Simionescu, 1984; Vorbrodt, 1988, 1994). The glycocalyx mainly comprises oligosaccharide moieties of plasmalemmal glycoproteins, and the notion that these fibrous macromolecules can influence permeability is encompassed in the ‘fibre-matrix model’ (Curry & Michel, 1980; Michel, 1988, 1996). In the fibre-matrix model the endothelial glycocalyx is proposed to act as a molecular sieve which can differentiate between molecules on the basis of their...
size. More recently, the role of EC surface charge has been incorporated into the model (Ghabriel et al. 1994; Rippe & Haraldsson, 1994). The proposition is that the charge characteristics of the endothelial glycocalyx and basal lamina may additionally influence discrimination between molecules on the basis of their charge. Furthermore, mutual repulsion between similarly charged groups within the glycocalyx would also contribute to the maintenance of an ordered fibre array (Ghabriel et al. 1994). On the luminal surface, the glycocalyx may affect the uptake of molecules into plasmalemmal vesicles or regulate transport through vesicular channels. Furthermore, a paracellular matrix, formed by arrays of junctional adhesion molecules, e.g. VE cadherin and PECAM-1, may similarly influence molecular size and charge restriction through the paracellular cleft. Evidence in support of this functional role for the glycocalyx is provided by experiments in which glycocalyx disruption, either by mild proteolytic treatment (Adamson, 1990) or polycation binding (Nagy et al. 1983), leads to a substantial increase in vascular permeability.

Since capillaries vary in their degree of passive diffusion, it is possible that this may also be reflected in quantitative and/or qualitative differences in the glycocalyx. The aim of the present study is to use a panel of lectins as probes to investigate global differences in the endothelial glycocalyx in vessels covering the range of permeability properties. Lectins are proteins, usually plant derived, which are carbohydrate binding and since they have a specificity for particular sugar residues, they are a powerful tool for glycoconjugate analysis.

**Materials and methods**

**Tissue processing and lectin labelling**

Five adult male Wistar rats (220–300 g) were anaesthetised with Sagatal and perfused transcardially with 0.1% glutaraldehyde-4% paraformaldehyde. The eyes, brain (4 mm coronal slice between the bregma and the lambda) and heart were removed and embedded in paraffin wax. Sections (7 μm) were labelled with the appropriate biotinylated lectin at a concentration of 250 μg/ml for 1 h at 37 °C followed by a streptavidin FITC conjugate (1:100 dilution) for 1 h. The lectins employed (Table 1) were obtained from Oxford Glycosystems, Abingdon, UK and Vector Laboratories, Peterborough, UK and the FITC conjugate from Sigma, Poole, Dorset, UK. The sections were observed and photographed using fluorescence microscopy. Controls consisted of omitting the lectin or using an appropriate inhibitory sugar in a preincubation step. The intensity of lectin labelling was assessed semiquantitatively on a 3 point scale (Table 2). Assessment was performed ‘blind’.

**Neuraminidase digestion**

Sections were incubated with neuraminidase (sialidase; New England BioLabs, Hitchin, Herts, UK) 1–10 U/ml overnight and then labelled with the lectins as above.

**Measurement of microvessel diameter**

Using an eyepiece graticule the minumum diameter of 30 vascular profiles was measured directly from

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**Table 1. Lectins employed**

<table>
<thead>
<tr>
<th>Source species and abbreviation</th>
<th>Major sugar specificities*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sambucus nigra</em> (SNA)</td>
<td>NeuNAc2–6Gal β-Gal</td>
</tr>
<tr>
<td><em>Maackia amurensis</em> (MAA)</td>
<td>NeuNAc2–3Gal β-Gal</td>
</tr>
<tr>
<td><em>Arachis hypogaea</em> (PNA)</td>
<td>Galβ1–3GalNAc β-Gal</td>
</tr>
<tr>
<td><em>Allomyrina dichotoma</em> (Allo A)</td>
<td>Triticum vulgare (WGA) β-GlcNAc NeuNAc</td>
</tr>
<tr>
<td><em>Canavalia ensiformis</em> (Con A)</td>
<td>α-GlcNAc α-Galα Galα Glc</td>
</tr>
<tr>
<td><em>Glycine max</em> (SBA)</td>
<td>β-GalNAc β-GalNAc</td>
</tr>
<tr>
<td><em>Ulex europaeus</em> (UEA-I)</td>
<td>α-Fucose</td>
</tr>
</tbody>
</table>

*NeuNAc, sialic (neuraminic) acid; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; Glc, glucose.

**Table 2. Lectin labelling of retinal, brain and myocardial microvessels***

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Retina</th>
<th>Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Maackia amurensis</em> (MAA)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Sambucus nigra</em> (SNA)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Con A</em></td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Triticum vulgare</em> (WGA)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Allo A</em></td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Glycine max</em> (SBA)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>PNA</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Ulex europaeus</em> (UEA-I)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Grading of labelling intensity: +++, strong; +++, moderate; +, weak; –, none.
micrographs of brain, retina and myocardium. The
mean vessel diameter was then calculated for each
tissue.

RESULTS

The results for lectin labelling of EC in brain, retina
and myocardium are illustrated in Table 2 and Figures
1 and 2. For quantitative purposes capillaries were
compared from the temporal cerebral cortex (mean
(± S.E.M.) vessel diameter 8.4 (±1.8) µm), the myocar-
dium of the ventricular wall (5.4 (±1.1) µm) and
the inner nuclear layer of the retina (6.3 (±1.7) µm).
In summary there was a general uniformity of EC
labelling, within and between tissues, for all the lectins
employed. However, myocardium EC were strongly
labelled with SNA and Con A while brain and retina
were moderately labelled. Also Allo A labelled
myocardial EC moderately but brain and retina
weakly. The labelling with the remainder of the lectins

Fig. 1. MAA (1a–c) and SNA (1d–f) labelling of microvessels (arrows) in brain (1a, 1d), retina (1b, 1e) and myocardium (1c, 1f). a, ×420; b, ×270; c, ×510; d, ×430; e, ×430; f, ×430.
Table 3. Lectin labelling of retinal, brain and myocardial microvessels* following neuraminidase digestion

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Brain</th>
<th>Retina</th>
<th>Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SNA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Con A</td>
<td>+ +</td>
<td>+ +</td>
<td>+ ++</td>
</tr>
<tr>
<td>WGA</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Allo A</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ ++</td>
</tr>
<tr>
<td>SBA</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PNA</td>
<td>+ +</td>
<td>+ + +</td>
<td>+ ++</td>
</tr>
<tr>
<td>UEA-I</td>
<td>–</td>
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<td>–</td>
</tr>
</tbody>
</table>

*Grading of labelling intensity: ++ + , strong; + + , moderate; + , weak; –, none.

was graded the same for myocardium, brain and retina. PNA did not label EC in any of the 3 tissues, but following neuraminidase (sialidase) digestion there was uniformly strong labelling (Fig. 2a,b). Labelling was also strongly increased with Allo A following neuraminidase digestion (Fig. 2 c,d), while labelling with MAA and SNA was eliminated. Labelling with the remainder of the lectins was unchanged after neuraminidase digestion (Table 3).

Control incubations in which an inhibitory sugar was introduced showed no labelling. Similarly the omission of the lectin in the incubation eliminated labelling.

DISCUSSION

The present study showed substantial sialic acid expression in the vasculature of each of the tissues studied. Labelling with both MAA and SNA was observed, indicating heterogeneity in the nature of the linkage of sialic acid to the preterminal sugar. MAA is specific for sialic acid in an α2–3 linkage to galactose (Knibbs et al. 1991) whereas SNA recognises the terminal sequence NeuNAcα2–6Gal (Taatjes et al. 1988). The appearance of PNA reactivity after neuraminidase digestion suggests the presence of Gal β1–3GalNAc capped by sialic acid. Taken together these data show that the sequences NeuNAcα2–3Gal β1–3GalNAc and NeuNAcα2–6Gal β1–3GalNAc are strongly expressed in the 3 types of microvessel. This uniformity was surprising in view of their different permeability characteristics. Myocardial capillaries are of the ‘leaky’ type, and typically most of the somatic vasculature are permeable to small hydrophilic molecules (Bundgaard, 1984). By contrast, capillaries in brain form a blood-brain barrier (BBB) and show a highly restrictive permeability to polar solutes (Brightman, 1992; Bradbury, 1993). This property is consistent with the maintenance of the homeostasis of the brain microenvironment which is an essential requisite for optimal neuronal function.

Retinal microvessels are also restrictive (Olsson & Kristenssen, 1973), but less so than the BBB. A recent comparison of the 2 vessels estimated a 4-fold higher rate of nonspecific permeability in the retina (Stewart & Tuor, 1994).

The present finding that leaky and barrier vessels show a similar qualitative and quantitative lectin binding profile implies some homogeneity in their fibre matrices. In view of the lack of any obvious correlation between glycocalyx composition and relative permeability, other permeability determinants in the vessels under investigation need to be considered. An important restrictive element is the paracellular cleft. Although the morphology of the cleft in continuous capillaries is basically similar, the degree of ‘tightness’ of the junctional strands which characterize the tight junction zone varies between vessels (Rippe & Haraldsson, 1994). High resolution goniometric tilting studies of endothelial paracellular clefts in somatic capillaries (Ward et al. 1988; Adamson & Michel, 1993) show a narrow (4 nm) membrane separation at the tight junction, with occasional 15–20 nm discontinuities within the junctional strands which allow the passage of ionic lanthanum (Bundgaard, 1984; Adamson & Michel, 1993). By contrast, tight junctional complexes of brain capillaries show fusion of the outer leaflets of adjacent plasma membranes and junctional strand discontinuities are absent (Schulze & Firth, 1992; Cassella et al. 1997). The results of the present study do not, however, contradict a gate role for the cleft matrix in capillaries with a patent paracellular pathway. The cadherin arrays which localise to the cleft are candidates for this role (Firth & Leach, 1996). Since the present LM study lacks the ability to adequately resolve differences in lectin domains in the paracellular region we cannot completely rule out the possibility that such differences do exist.

A second morphological difference between tight and leaky capillaries is in the relative numbers of plasmalemmal vesicles. Although still controversial, there is compelling evidence that these structures, either by transendothelial shuttling or via vesicular channel formation, are involved in the nonspecific transport of macromolecules across capillary walls (Michel, 1996). Vesicular profiles are very common in somatic capillaries (Simionescu & Simionescu, 1984; Simionescu et al. 1985). By contrast, these structures are relatively sparse in capillaries of brain and retina (Stewart & Tuor, 1994), suggesting that under
normal conditions BBB vessels display limited passive transcellular transport. Moreover, this may also be a factor in their restricted permeability to macromolecules (Brightman, 1992; Bradbury, 1993). However, there is evidence that macromolecular permeability across capillary beds in general is charge-dependent (Rippe & Haraldsson, 1994). This is also true of brain, for example the cationization of albumin increases its transport at the BBB in vivo (Shimony-Hophy et al. 1991) and across brain EC in vitro (Vorbrodt & Trowbridge, 1991). The anionic nature of the fibre matrix (confirmed in the current study) may therefore be a contributing factor in charge selectivity in all the vessels under investigation.

The striking uniformity in lectin labelling between BBB and non-BBB capillaries suggests the possibility that many of the lectin ligands which occupy luminal and abluminal EC surfaces are concerned with a
common set of cellular housekeeping roles. Although the oligosaccharide chains of membrane glycoproteins contain relatively few of the large numbers of monosaccharides found in nature, sequence diversity and variation in glycosidic linkage confers a broad functional specialisation and there is now clear evidence that these moieties are important recognition sites in many cell: cell and cell: matrix interactions (Paulson, 1989; Cooper, 1997). In vascular EC luminal ligands could include various coagulation factors (Varki, 1993) and molecules involved in leucocyte signalling and adhesion (McEver, 1992). Abluminally, lectin binding sites may be associated with members of the integrin family of adhesion molecules (e.g. α2β1, α5β1) which mediate EC attachments to the extracellular matrix (Caveda et al. 1994).

In summary, the relative contribution of glycocalyx heterogeneity in the permeability of different vascular endothelia remains to be established. Although there was no clear evidence of any differences in the global pattern of lectin labelling, between BBB and non-endothelia, it is possible that lectin ligands may differ in the region of the paracellular cleft which is known to show considerable structural specialisation in different vascular beds.

REFERENCES


