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Identification of lectin binding sites in the rat brain

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Lectins belong to a class of proteins or glycoproteins able to bind carbohydrates. The study reported here describes the identification of lectin-binding sites in the adult rat brain. The results indicate that among the 31 lectins utilized, eight show a specific positive reaction with neurons. Staining was also observed with other cerebral structures such as myelin, leptomeninges, choroid plexus and capillaries. Lectins are, therefore, an important histochemical tool and can be easily and reliably used for the identification of cells and cerebral structures in the adult rat brain.

Keywords: lectins, receptors, rat brain, glycosylation

Abbreviations: Gal, galactose; Fuc, fucose; Man, mannose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; NeuNAc = sialic acid.

Introduction

Lectins represent a class of multivalent carbohydrate-binding proteins or glycoproteins. They were first, characterized in plants, having cell-agglutinating properties, but being distinct from enzymes and antibodies [1]. Lectins were also found in animal tissues associated with the cell surface, cytoplasm and cell nucleus [2]. Endogenous lectins and glycoconjugates are assumed to be involved in cell regulation and differentiation [3]. The interaction between lectins and glycoconjugates is of paramount importance to both intracellular recognition and cell-cell adhesion phenomena. Lectins have been employed in histopathology to identify abnormal glycosylation in mammalian endothelium [4], in Batten's disease [5], in the pathological features of Alzheimer's disease [6], as well as in other pathological conditions. In addition, several reports have described lectin 'receptors' in mouse brain embryo [7], in rabbit temporomandibular joint disc [8], in neuroblastoma cells [9], in primary olfactory neurons from rat nervous system [10], in canine blood-brain barrier [11], in the vessel beds of the rat eye [12], in frog brain [13], in the myelin from mice [14–15], and in canine cerebral cortex [11]. In Alzheimer's disease, for instance, we have recently reported that senile plaques, as well as neurofibrillary tangles, characteristic of the pathology of this devastating syndrome, are differently-glycosylated [6]. Furthermore, we have previously found that toxicological and pharmacological treatment of neuroblastoma cells can modify the histochemical expression of lectin-binding glycans [16]. The aim of the present study was to identify lectin binding sites in normal rat brain that could be used to study the variations of glycans in brain cellular structures which may change as a consequence of toxicological or pharmacological treatment.

Materials and methods

Brain tissues from ten adult Wistar rats were fixed in 10% (by vol) formalin in 10 mM saline phosphate buffer (PBS), pH 7.5, for 48 h before embedding in paraffin. Sections (5 μm thickness) were deparaffinized and hydrated according to standard procedures [17]. Sections were treated for one hour with 50 mM NH_4Cl in 10 mM PBS, pH 7.2, in order to block aldehyde groups and washed

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with PBS. Endogenous peroxidases were blocked by incubation with 3% (by vol) H_2O_2 in PBS for 10 min, and washed again in PBS for 5 min. Sections were then covered with 3% (by vol) bovine serum albumin in PBS for 30 min, then incubated separately with one of the lectins (Sigma) listed (Table 1). Lectins were applied in solution for 1 h at a concentration of 10 $\mu g\,ml^{-1}$ in 20 mM Tris buffer, pH 7.4, in a wet chamber. Sections were washed in PBS, followed by incubation with streptavidin-biotin-peroxidase (Pierce) for 30 min and washed again with PBS. The chromogenic reaction with horseradish peroxidase (HRP) was obtained in Tris-HCl buffer, pH 7.2, 0.1 M in the presence of 3,3′-diaminobenzidine, 10 mM $CaCl_2$ and 0.3% (by vol) H_2O_2. Sections were washed, counterstained with hematoxylin, rinsed in Milli-Q water, dehydrated in a series of steps from aqueous alcohol to absolute alcohol and then to xylene, and were mounted with clearium medium (Surgipath). A control was routinely performed using a solution of lectin with its sugar antagonist, following the procedure described above [6]. In preliminary experiments, controls were performed by omitting the lectin. Comprehensive data regarding the interactions of each of the 31 lectins utilized in our study with rat brain specimens are presented in Table 2. Staining intensity, was evaluated and expressed as: strong (+++), moderate (++) weak (+), very weak (±) and negative (−).

Results and discussion

Table 2 summarizes the staining intensity of the reactions between lectins and various brain structures. Certain cell types and structures are considered in detail. There are neurons, myelin, choroid plexus and capillaries. Only eight lectins showed positive reactions with neurons. The reactions with neuronal membrane are summarized in Table 3.

A positive reaction with myelin was observed with 10 lectins (BPA, HPA, JCA, PAA, PsAA, PTA, SBA, SJA, SNA, UEA I). A typical example of the interaction of myelin with lectin is shown for BPA in Fig. 1D.

Table 1. Lectins utilized in the identification of lectin binding sites in the rat brain

Acronym	*Lectin origin*	*Specificity*
1. AAnA	Anguilla anguilla	α-L-Fuc
2. ABiA	Agaricus bisporus	β-D-Gal(1-3)-D-GalNAc
3. APA	Abrus precatorius	D-Gal
4. BPA	Buahunia purpurea	β-D-Gal(1-3)D-GalNAc
5. BSA-I	Bandeiraea simplicifolia	α-D-Gal; α-D-GalNAc
6. CFA	Codium fragile	D-GalNAc
7. ConA	Concanavalia ensiformis	α-D-Man; α-D-Glc
8. DBA	Dolichos biflorus	α-D-GalNAc
9. DSA	Datura stramonium	$(D\text{-}GlcNAc)_2$
10. ECL	Erythrina cristagalli	β-D-Gal-(1-4)D-GlcNAc
11. ECO	Erythrina corallodendrum	β-D-Gal-(1-4)-D-GlcNAc
12. EEA	Euonymus europaeus	α-D-Gal(1-3)D-Gal
13. HPA	Helix pomatia	α-D-GalNAc; D-GlcNAc
14. JCA	Artocarpus integrifolia	α-D-Gal; Gal-β-(1-3)GalNAc
15. LCA	Lens culinaris	α-D-Man
16. LEA	Lycopersicon esculentum	$(D\text{-}GlcNAc)_3$
17. LPA	Limulus polyphemus	NeuNAc; D-GlcNAc
18. PTA	Lotus tetraglonolubus	L-Fuc-α-(1-2)Gal-β-(1-)[L-Fuc-α(1-3)]GlcNAc
19. MGA	Mycoplasma galliseptum	Glycophorin
20. PAA	Phytolaca americana	$(-D\text{-}GlcNAc)_3$
21. PHA-E	Phaseolus vulgaris	Bisected, bi- triantennary N-glycans
22. PNA	Arachis hypogaea	β-D-Gal(1-3)D-GalNAc
23. PsAA	Pseudomonas aeruginosa	D-Gal
24. PTA	Psophocarpus tetragonolobus	D-GalNAc; D-Gal
25. RCA	Ricinus communis	D-GalNAc; DGal
26. SBA	Glycine max	α-D-GalNAc
27. SJA	Sophora japonica	β-D-GalNAc
28. SNA	Sambucus nigra	NeuNac-α-(2-6)Gal
29. UEA-I	Ulex europaeus	L-Fucα-Galβ1-4GlcNAc
30. WFA	Wisteria floribunda	D-GalNAcα1-6Galβ1-
31. WGA	Triticum vulgaris	$(-D\text{-}GlcNAc\beta1\text{-})_2$; NeuNAc

Table 2. Summary of the staining intensity of the reactions between lectins and various brain structures

Lectin	*Neurons*		*Glial cells*		*Capillaries*			
	Cytopl.	*Nucl.*	*Astroc.*	*Oligodend.*	*Luminal*	*Ablum.*	*Leptomen.*	*Chor. plex.*
ABiA	–	–	–	++	++	–	++	++
AAnA	–	–	–	–	–	–	–	–
APA	++	–	–	+	+	–	+	+
JCA	+++	–	–	++	–	+	++	++
BPA	–	–	–	+++	–	–	–	–
BSA-I	–	–	–	±	–	–	–	–
CFA	–	–	–	+	–	–	–	–
ConA	+++	–	–	++	+++	–	+++	+++
DBA	++	–	–	–	–	–	–	–
ECL	–	–	–	–	–	–	–	–
ECO	–	–	–	+	–	–	–	–
EEA	–	–	–	+	–	–	–	–
HPA	+++	–	–	+++	–	–	–	–
LCA	–	–	–	++	+++	–	+++	+++
LEA	–	–	–	++	+	–	++	++
LPA	–	–	–	–	–	–	–	–
LTA	–	–	–	–	–	–	–	–
MGA	–	–	–	–	–	–	–	–
PAA	–	–	–	+	++	–	–	–
PHA-E	–	–	–	–	–	–	–	–
PNA	–	–	–	+++	–	–	–	–
PsAA	–	–	–	+	++	–	–	–
PTA	–	–	–	++	–	–	–	–
RCA	–	–	–	++	+++	–	+++	+++
SBA	+++	–	–	+	–	–	–	–
SJA	–	+	–	++	–	–	–	–
SNA	–	–	–	+++	++	–	+	+++
DSA	–	–	–	++	+++	–	+++	+++
UEA-I	–	–	–	+++	–	–	–	–
WFA	+	–	–	+	–	–	+	+
WGA	–	–	–	++	+++	–	+++	+++

Table 3. Summary of the staining intensity of the reactions between lectins and the neuronal membrane

Lectin	*Observations*
APA	Positive reaction on the neuronal cytoplasmatic membrane of the intermediate layer of the brain cortex.
ConA	Cytoplasm of cortical neurons appeared coloured in a granular fashion.
DBA	A few neurons of the intermediate layer of the parietal cortex reacted positive, with focal staining (Fig. 1A) Magnification × 1050.
HPA	Cytoplasm and the external membrane of the intermediate layer of the frontal and parietal cortex reacted positively (Fig. 1B) Magnification × 1050.
JCA	Strong positive reaction of the external membrane of the Purkinje cells.
SBA	i) Strong positive reaction of the external membrane of neurons belonging to the intermediate layer of the frontal cortex (Fig. 1C). ii) Staining on the perikaryon.
SJA	This is the only lectin that stains the nucleoplasm of neurons from the parietal and frontal cortex in a granular fashion.

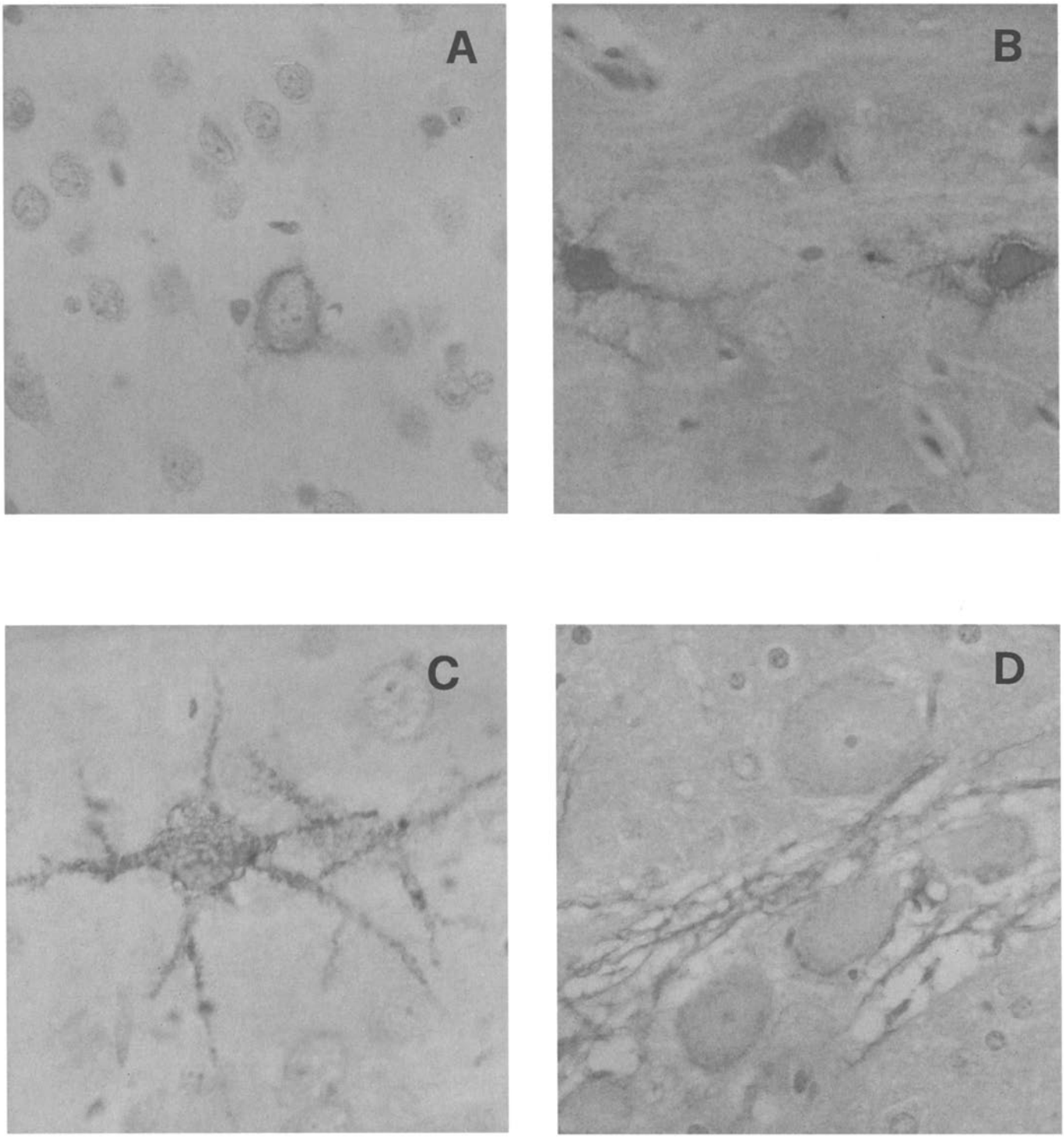
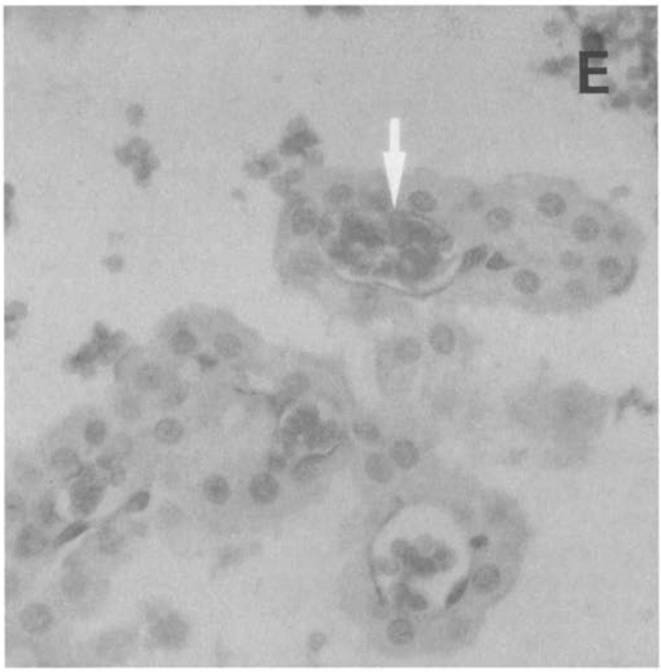
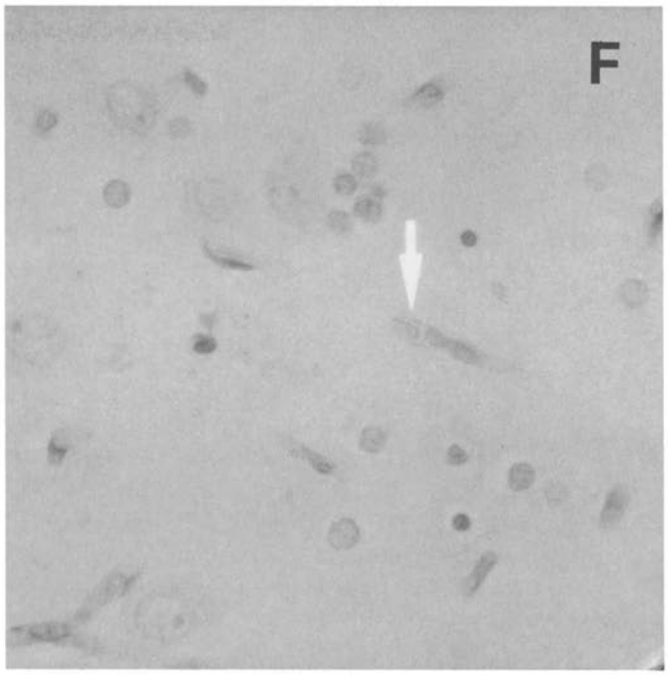


Figure 1. Light microscopy of brain cells or other cerebral structures stained with different lectins. (A) DBA. Focal positive reaction in a few neurons belonging to the intermediate layer of the parietal cortex (magnification × 1050). (B) HPA. Positive reaction of the external membrane and cytoplasm of neurons in the intermediate layer of the frontal and parietal cortex (magnification × 1050). (C) SBA. Strong positive reaction of external membrane and cytoplasm of neurons in the intermediate layer of frontal cortex. (magnification × 1050). (D) BPA. Positive reaction of myelin sheet from oligodendrocytes (magnification × 1050). (E) LCA. Positive reaction of the luminal side as well as epithelial cells forming the choroid plexus marked by an arrow. In E and F, note that erythrocytes are strongly stained (magnification × 1050). (F) PAA. Brain cortex: moderate staining of luminal side of microcapillaries marked by an arrow (magnification × 1050).





Interaction with choroid plexus was observed only on the luminal side of the epithelial cells forming this structure (Table 2 and Fig. 1E for a typical example). Many of the lectins used were able to discriminate the luminal side of capillaries (see Fig. 1F for example). Only JCA has a weak reaction with the abluminal side.

Brain tissue is largely composed of membraneous structures, including nerve-endings, axons, dendritic and glial processes etc., and these are universally glycosylated. Our study indicated that, under appropriate conditions, lectin binding sites could be identified, and these can be used as a valuable tool to distinguish different cerebral structures. Our findings show that only SJA, which recognizes β-D-GalNAc was able to interact with the nuclear membranes of brain cells. Few previous reports, describe the existence of glycoconjugates in the nucleoplasm of cells with O-linked glycan [18].

Lectin staining of neuronal membranes seems to be unevenly distributed, and micropearl-shaped features, probably attributable to synaptic axosomatic junctions, were observed. Lectins giving a positive reaction with neurons were those that recognize Gal, GalNAc and Man. Brain coronal sections offered the best material for histochemical analysis, showing particularly the stereotaxic interval between intraneural plate 7.70 mm-bregma 1.30 mm and intraneural plate 7.20 mm-bregma 1.80 mm.

All lectins, except PTA, showed a correlation between lectin staining for erythrocytes and the luminal wall of the leptomeningeal vessels. Choroid plexus and leptomeninges showed similar staining patterns and this could reflect the contact of both structures with the cerebrospinal fluid.

Neuronal cytoplasm was strongly stained by ConA, HPA, JCA, SBA and moderately by APA and DBA. In contrast, the only lectin able to stain, even if weakly, the nucleoplasm of cortical neurons was WFA, which recognizes D-GalNAcα1-6Galβ- [19].

Astrocytes showed negative results with all 31 lectins used. Oligodendrocytes stained strongly with BPA, PNA, HPA, SNA and UEA-1 which recognize β-D-Gal(1-3)D-GalNAc, β-D-Gal (1-3)D-GalNAc, GalNAc, α2,6-linked NeuNac, and α-L-Fuc respectively.

The luminal side of parenchymal capillaries were strongly stained by LCA, which recognizes α-D-Man and various complex, non-bisected N-glycans, and moderately stained by DSA which recognizes D-(GlucNAc)$_2$. The abluminal side of the capillaries showed a weak reaction only with JCA, which recognizes α-D-Gal and β-DGal(1-3)D-GalNAc [20].

It is been reported that PHA binds to frog brain and this lectin is utilized to study axonal transport elements, where a lectin receptor, both in anterograde and retrograde transport, has been observed [13]. This is in contrast to mammalian nervous systems where the PHA receptor involved in transport was only observed in the retrograde direction [13]. RCA, which recognizes D-galactosyl residues, stained capillaries from canine cerebral cortex intensely [11]. This lectin was found to bind to the luminal membrane of endothelial cells. Strong positive reaction was also observed in canine brain

vessels with PHA-E, PSA and LCA that recognize N-glycans, D-Gal and Man. Furthermore, the distribution of lectin binding sites differed between capillaries and arterioles, as has been reported from other laboratories [21].

Conclusion

Our study shows that lectins bind selectively to particularly rat brain structures, with the possibility that this can be used to discriminate between cell types on the basis of different glycosylation patterns. This could be useful to study modified glycosylation sites in the brain in certain pathological situations.

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