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Proteoglycans in the nervous system

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Proteoglycans are ubiquitous cell-surface and secreted glycoproteins that are involved in diverse cellular behaviors. The identities of several nervous system proteoglycans, including many of the major species in the mammalian brain, have recently come to light. In addition, recent studies have given new insights into the roles of proteoglycans in nervous system development and function.

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Introduction

Proteoglycans (PGs) are found on the surfaces of all adherent cells, within intracellular vesicles, and in virtually all extracellular matrices (ECMs). They are evolutionarily ancient molecules, and play functional roles in the biology of growth factors, extracellular proteolysis, cell adhesion, lipoprotein metabolism, and virus entry into cells, as well as structural roles in maintaining the physical and mechanical properties of ECMs [1,2•,3,4].

Although many basic characteristics of PGs — their number, their structures, the exact nature of the functions they perform — are still slowly emerging, great progress has been made in recent years. With this recent burst of activity has come increasing recognition of the significance of PGs by neurobiologists, and increasing interest in the postulated roles PGs play in the nervous system. Some investigators have isolated monoclonal antibodies against nervous system molecules that have turned out to be PGs. Other investigators have become intrigued by the fact that many of the molecules that are thought to influence neuronal and glial cell behavior *in vivo*, especially during development, bind PGs. In the last few years, direct assaults on determining the structures of central nervous system (CNS) PGs have been undertaken by several groups. The purpose of this article is to review some of these recent results, and place them into the wider context of what PGs are, and how they are thought to function.

What are PGs?

A protein is called a PG if it contains a covalently attached glycosaminoglycan (GAG). GAGs are linear polysaccharides, typically 20–200 sugars in length, which are usually attached via a characteristic linkage region to serine residues. GAGs are built by the sequential addition of identical disaccharide units onto this linkage region. Only three types of disaccharide may be used, giving rise to three families of GAGs: the heparin/heparan family [D-glucuronic acid $\beta(1\rightarrow4)$ D-*N*-acetyl glucosamine $\alpha(1\rightarrow4)]_n$; the chondroitin/dermatan family [D-glucuronic acid $\beta(1\rightarrow3)$ D-*N*-acetyl galactosamine $\beta(1\rightarrow4)]_n$; and the keratan family [D-galactose $\beta(1\rightarrow4)$ D-*N*-acetyl glucosamine $\beta(1\rightarrow3)]_n$. The sugars of most GAGs are further chemically modified, typically in a sporadic fashion throughout the chain, by *O*-sulfation, *N*-deacetylation followed by *N*-sulfation, and/or epimerization (isomerization) of glucuronic acid to iduronic acid. Subsequently, GAGs are referred to as heparin, heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS) or keratan sulfate (KS). The heparin/HS distinction and the CS/DS distinction only reflect differences in level of modification (i.e. heparin is more highly modified than most HS species; DS contains much more iduronate than CS). As each disaccharide in a GAG chain may be modified to a different degree, the large scale structures of GAGs can be exceedingly complex (e.g. in HS, which can be modified in up to five ways, a hexasaccharide can theoretically have over 30,000 possible chemical structures).

Products of several gene families, including secreted and membrane-inserted polypeptides, act as the core proteins of major PGs (Table 1). Some bear as few as one GAG chain, whereas others have over a hundred. Although the signals that specify whether a serine residue will bear a GAG are partially understood [2•], it is not known what controls the type of GAG synthesized: examples exist of cores that always bear one type of GAG, cores that bear different GAGs at different sites, and cores that bear different GAGs depending on the cell type in which they are expressed.

Abbreviations

CNS—central nervous system; **CS**—chondroitin sulfate; **DS**—dermatan sulfate; **ECM**—extracellular matrix; **FGF**—fibroblast growth factor; **GAG**—glycosaminoglycan; **HS**—heparan sulfate; **KS**—keratan sulfate; **NCAM**—neural cell adhesion molecule; **NgCAM**—neuron-glial cell adhesion molecule; **PG**—proteoglycan.

Table 1. Cloned PG core proteins.[a]

Cell-surface PGs	
	Syndecan family
	Syndecan (Syndecan-1)
	Fibroglycan (Syndecan-2)
	N-Syndecan/Syndecan-3
	Ryudican/Amphiglycan/Syndecan-4
	Glypican family
	Glypican
	Cerebroglycan
	NG-2
	'Part-time PGs'[b]
ECM PGs	
	Aggrecan family
	Aggrecan
	Versican
	Neurocan
	Small, interstitial PG family
	Decorin
	Biglycan
	Fibromodulin
	Lumican
	Perlecan
	Type IX Collagen
Intravesicular PGs	
	Serglycin
	SV2

[a]Only shown are the obligate PG core proteins, i.e. those that invariably bear GAG chains. A small number of other cell-surface proteins bear GAG chains in some cells, but not others. [b]These 'part-time' PGs include CD44 and the type III transforming growth factor (TGF)-β receptor (reviewed in [1,2•,53]).

Cell surface PGs of the CNS

Early progress toward identifying cell surface PGs of the brain was made by Margolis' group, who detected a single major HSPG in adult brain membranes [5]. Later, Herndon and I [6] found evidence for CSPGs and other, less abundant, HSPGs in adult brain membranes, as well as additional major HSPGs that are present only during development. In the past year, the core proteins of several of these have been identified.

Glypican

Glypican was first identified as a surface HSPG core protein of human fibroblasts [7]. The mature polypeptide is 53 kDa and is anchored in the plasma membrane by covalently attached glycosylphosphatidylinositol. Both the adult brain HSPG identified by Klinger *et al.* [5], and brain HSPG M12 identified by us [6], are the rat form of glypican ([8]; ED Litwack, CS Stipp, A Kumbasar, AD Lander, unpublished data). *In situ* hybridization studies in the adult brain and spinal cord indicate that glypican mRNA is expressed primarily, if not exclusively, by projection neurons in many, but not all parts of the CNS (ED Litwack, CS Stipp, A Kumbasar, AD Lander, unpublished data) (see Table 2). In the embryo, glypican is also strongly expressed in ventricular zones (regions undergoing neural precursor proliferation) throughout the neuraxis (Fig. 1).

Cerebroglycan

Cerebroglycan, previously called PG M13 [6], is an HSPG with a ~58 kDa core protein, and was first detected in the embryonic and newborn — but not adult — rat brain. Like glypican, it is glycosylphosphatidylinositol-anchored. In fact, glypican and cerebroglycan define a family of lipid-anchored HSPG cores, based on amino acid sequence similarity (CS Stipp, ED Litwack, AD Lander, unpublished data) (see Table 2). *In situ* hybridization studies indicate that cerebroglycan is transiently expressed by postmitotic neurons throughout the CNS (Fig. 1). Evidently, cerebroglycan mRNA appears in neurons shortly after terminal mitosis and disappears after neuronal migration and axon growth have been completed. Interestingly, cerebroglycan is not expressed outside the nervous system.

N-syndecan

N-syndecan (or syndecan-3) is one of four members of the syndecan family of transmembrane core proteins (Table 1). These polypeptides have short (~34 amino acids) cytoplasmic domains that are highly conserved among all family members, and overall sizes varying from 20 kDa (syndecans-2 and -4) to ≥ 42 kDa (syndecan-3). Their extracellular domains are poorly conserved among the different family members, or even for the same syndecan in different mammalian species. *N*-syndecan was cloned by Carey *et al.* [9••], who identified it in rat Schwann cell membranes (see Table 2). High levels of *N*-syndecan mRNA are also found in neonatal rat brain, as well as in many sites outside the nervous system. Expression of this molecule in rat brain peaks at birth, declining to undetectable levels thereafter. Early immunohistochemical studies suggest that this PG is associated with fiber tracts, but it is not yet known whether its source is neuronal or glial.

Syndecan-2

Syndecan-2, also known as fibroglycan, another member of the syndecan family, has not yet been isolated from the brain, but its mRNA has been found there (see Table 2). Based on electrophoretic behavior, syndecan-2 may correspond to brain PG M14 [6].

Table 2. PGs of the mammalian CNS.[a]

Name	Family	GAG	CNS Expression
Syndecan-3	Syndecan	HS	Transiently expressed in perinatal brain; widespread [9••]
Glypican[b]	Glypican	HS	Neuroepithelium; certain adult projection neurons [8][c]
Cerebroglycan[b]	Glypican	HS	Transiently expressed by newly post-mitotic neurons[d]
NG-2	NG-2	CS	O-2A progenitors [10]
Syndecan-2	Syndecan	HS	Unknown [2•]
Neurocan (1D1)	Aggrecan	CS	White matter of developing cerebellum; molecular layer of adult cerebellum. Mostly intracellular in adult [12••,18]
Versican	Aggrecan	CS	White matter [13•]
Aggrecan	Aggrecan	CS	Embryonic chick brain [14•]
Cat-301	Aggrecan?	CS	Subsets of neurons, cerebellum and spinal cord [15•,20]
PG-T1	?	CS	Widespread [16••,17•]
3H1	?	CS/KS	Similar to neurocan [18]
3F8	?	CS	Concentrated in molecular layer of developing and adult cerebellum [18]
6B4	?	CS	Cerebellar and brainstem projection neurons [19•]
Unnamed	?	HS	Transient, in CNS fiber tracts [26••]
SV2 antigen	SV2	KS	Synaptic vesicles [27••]

[a]PGs are referred to by the names of their core proteins, and are grouped according to whether they are cell surface, extracellular matrix/soluble, or intravesicular molecules (see text). In many cases, information on CNS distribution has been based on the examination of only a few brain regions, and is therefore incomplete. [b]Data on distribution of glypican and cerebroglycan are based on *in situ* hybridization; most other data were obtained using antibodies. [c]ED Litwack, CS Stipp, A Kumbasar, AD Lander, unpublished data. [d]CS Stipp, ED Litwack, AD Lander, unpublished data.

NG2

NG2 is a transmembrane CSPG with a 300 kDa core protein [10]. In the brain it is associated with a population of glial precursor cells, the O-2A progenitors (see Table 2), that give rise to oligodendrocytes and a type of astrocyte. The very large core protein of NG2 suggests that it may serve functions other than just bearing CS chains. One such function appears to be the binding of type VI collagen [11].

ECM and 'soluble' PGs of the CNS

Many PGs can be extracted from the brain using physiological buffers without detergent; others require high salt or denaturing conditions. Although it has been argued that some of these molecules may reside in the cytoplasm of cells, most are probably loosely associated with the ECM.

Most of the PGs in these categories contain CS as their major GAG. Neurocan, a recently cloned CSPG, has a 136 kDa core protein, and contains ~3 CS chains [12••]. Its protein sequence places it in a family with aggrecan — the major ECM PG of cartilage — and versican, an ECM PG first found associated with fibroblasts. Like these other PGs, neurocan binds the ECM polysaccharide hyaluronic acid via a protein domain that is highly conserved in all three family members. Recent evidence suggests that versican and aggrecan are themselves expressed in the human and chicken brain, respectively [13•,14•]. The Cat-301 antigen is yet another large brain CSPG that binds hyaluronic acid, and immunological evidence suggests that it is related to aggrecan [15•]. One additional hyaluronic acid-binding CSPG, the T1 antigen, has been identified in brain, but at least the hyaluronic acid-binding region of this molecule is apparently unrelated to those of the aggrecan family [16••,17•]. Still other brain CSPGs have been identified with monoclonal antibodies, and remain to be fully characterized [14•,18,19•].

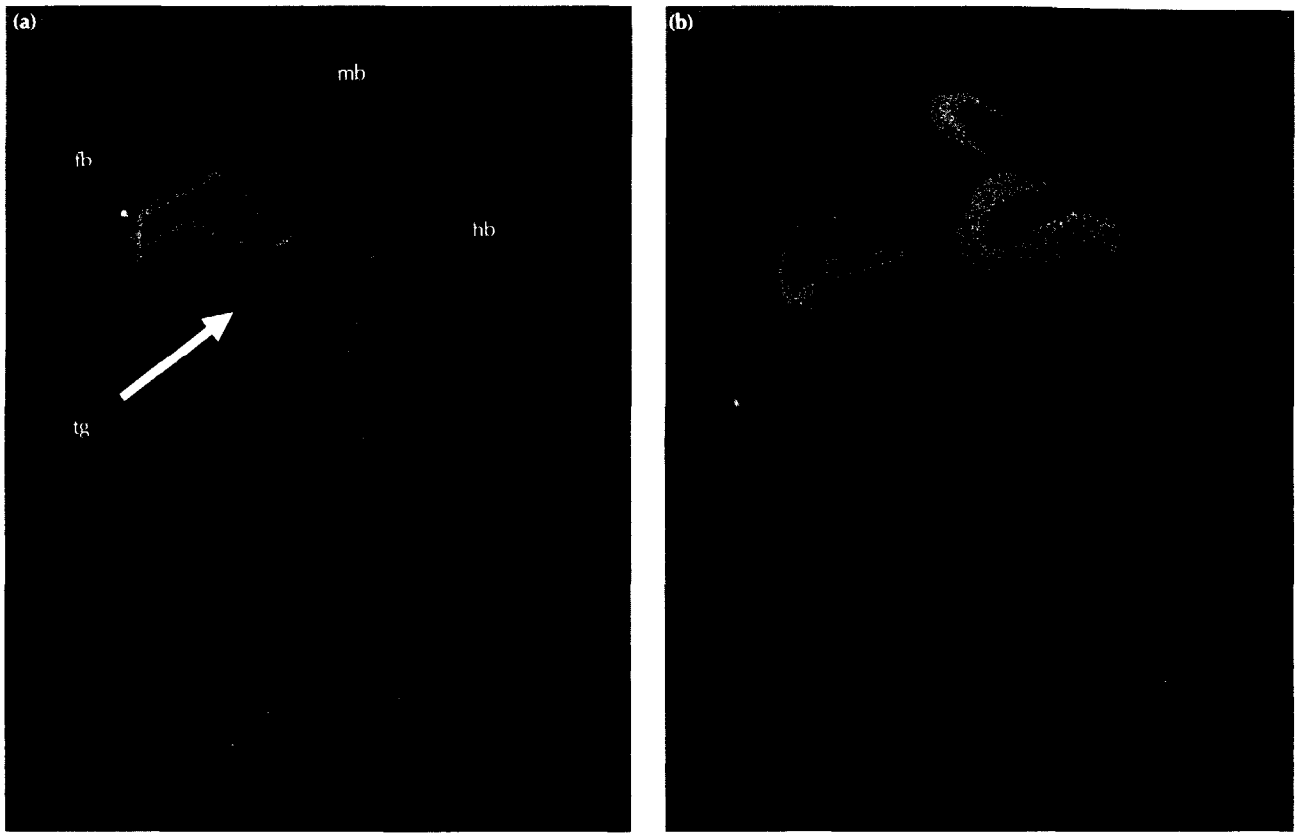

Fig. 1. Expression of glypican and cerebroglycan in the rat embryo. Adjacent sections of embryonic day 14 rats were hybridized with radiolabeled RNA probes specific for **(a)** glypican and **(b)** cerebroglycan mRNA. The images are reverse contrast prints of the resulting autoradiograms. Glypican expression is found throughout the embryo, but is particularly strong in the ventricular zones of the developing CNS. In contrast, cerebroglycan mRNA, which is found only in neural tissue, is not detected in ventricular zones but is found in the layers of immature neurons that form around those zones. In the adult brain, glypican is expressed by subpopulations of neurons, whereas cerebroglycan is absent. fb—forebrain; mb—midbrain; hb—hindbrain; tg—trigeminal ganglion.

The distributions of these CSPGs vary from remarkably uniform throughout the brain (PG T1) to remarkably cell type- and developmental stage-specific. For example, Cat-301 appears around certain subsets of neurons only after activity-dependent critical periods in their development [20]. Another is transiently expressed during axon outgrowth by several types of neurons involved in the cerebellar mossy fiber system [19•].

As information on the distribution of these CSPG and CS/KSPG core proteins accumulates, so has information on the distribution of different types of CS and KS chains. Several investigators have observed remarkable cell-type specificity in the binding of anti-CS and anti-KS monoclonal antibodies to brain sections (e.g. [21,22]). During cerebral cortex development, CS is found in the early proliferative neuroepithelium, then later in the marginal zone and subplate regions [23]. With the exception of the subplate, many of the locations of CS expression during development correlate with sites where axons do not grow. For example, CS, as well as KS, are strongly expressed in the roof plate of the spinal cord [24,25].

Recently, a report of an HSPG in brain ECM appeared [26••]. This molecule is found in basal laminae outside of and surrounding the chicken brain, but is also expressed transiently in many developing CNS axon tracts. The core protein size (250 kDa) and basal laminar distribution of this PG are reminiscent of perlecan, a major basement membrane PG, but perlecan itself is not found in CNS axon tracts.

Synaptic vesicle PGs

It has long been known that a PG is a major component of synaptic vesicles isolated from the electric organs of fishes. This PG was recently shown to be a transmembrane KSPG, and appears to be involved in acetylcholine transport into vesicles [27••]. Immunochemical data suggest that this molecule is present in many other types of synaptic vesicles, and might therefore play an important general role in transmitter uptake.

Roles of PGs in the nervous system

Insights into the functions of PGs in the nervous system have come by many routes, direct and indirect, and many of the conclusions are still somewhat preliminary. Highlights of what has been learned are summarized below.

The functions of a family of growth factors are dependent on PGs

All members of the fibroblast growth factor (FGF) family bind GAGs of the heparin/HS class, and apparently must do so to be biologically active [28,29]. Recent studies support a model in which cell-surface HSPGs bind both FGFs and FGF receptors simultaneously, facilitating their interaction [30]. It is known that at least three FGFs — FGF-1, -2 and -5 — are expressed in the nervous system and exert trophic effects on several classes of neurons [31–34,35••]. Recently, Nurcombe *et al.* [35••] have suggested that differences in the type of HS carried by a single core protein can render early neuroepithelial cells selectively responsive either to FGF-1 or to FGF-2. This proposition is supported by evidence in other systems that HS structure can impart specificity to HSPG function (e.g. [36,37•,38,39•]).

The kinetics of action of a family of protease inhibitors are dependent on PGs

The structurally related molecules antithrombin III, heparin cofactor II, and protease nexin I all bind and inactivate certain serine proteases (e.g. thrombin) much more rapidly when appropriate GAGs are present. To a large extent, GAGs act by simultaneously binding both protease and protease inhibitor, confining them to the same locality and thereby facilitating their interaction [38]. Of interest to neurobiologists, protease nexin I is abundantly expressed in the CNS, and is thought to regulate neurite outgrowth and neuronal migration [40].

Cell surface PGs participate in establishing cell–cell and cell–ECM contacts

Although cell surface PGs can apparently be the sole receptors for attachment to certain substrata [37•], PGs usually facilitate interactions mediated through other receptors, such as integrin-dependent cell attachment to ECM molecules [41], and neural cell adhesion molecule (NCAM)-dependent cell–cell adhesion [42,43]. A recent study suggests that cell surface HSPGs are especially important for the interaction of neural cells with fibronectin [44•]. As ECM and cell adhesion molecules are thought to provide important navigational cues to growing axons, the involvement of PGs with such molecules suggests a potential role for PGs in axon guidance. Recent studies in insects support this idea [45••].

ECM PGs regulate cell–cell and cell–matrix interactions

The core protein of at least one PG, perlecan, supports integrin-mediated cell attachment [46]. In contrast, several PGs inhibit the biological activities of ECM and cell adhesion molecules, at least *in vitro*. For example, adsorbed CSPGs or CS/KSPGs can render culture substrata inhospitable for neurite growth [24,25]. Soluble CSPGs from rat brain also inhibit neurite outgrowth by PC12 cells [47]. Neurocan and the 3F8 CSPG of rat brain (but not aggrecan) inhibit homophilic NCAM and neuron-glial cell adhesion molecule (NgCAM)-binding [48•]. A HSPG released by Schwannoma cells specifically blocks the neurite outgrowth-promoting activity of laminin [49]. In some of these cases, the GAG chains of the PGs are required for these actions [24,25,49]; in others they are not [47,48•]. It is not yet known whether these phenomena are direct actions of PGs on neurons, or reflect effects of PGs on the physical characteristics of the culture substratum, so caution must be used in extrapolating these results to *in vivo* settings. Nonetheless, the distributions of some CSPGs are consistent with a 'barrier' function *in vivo* (see above). For example, in the developing retina a receding wave of CS expression marks a front of centripetally directed axons, suggesting that axons are guided by their avoidance of CS. Intriguingly, a CS-degrading enzyme disrupts the timing and direction of retinofugal axons in the developing rat retina [50••].

PGs are involved in the assembly of ECM, and act as binding sites for molecules that associate with the ECM

PGs bind virtually every major ECM component. In addition, molecules such as growth factors (e.g. FGFs) and enzymes (e.g. synaptic acetylcholinesterase) are often immobilized in ECMs through interactions with HSPGs [1,51]. The importance of PGs in ECM structure and function is illustrated by a muscle cell line that is defective in GAG biosynthesis [52•]. This cell line produces an abnormal basal lamina and, probably as a consequence, fails to form acetylcholine receptor clusters. The cells also fail to form such clusters in response to agrin, a GAG-binding ECM molecule that potently induces receptor clusters on normal muscle cells, and is thought to be involved in synaptogenesis *in vivo*.

Conclusions

Although much still needs to be learned about nervous system PGs, the identities of many of the major species in the brain are now known. Tracking down the functions of these molecules will probably not be easy. Their biological activities are likely to reside in their capacity to regulate, possibly in subtle ways, the functions of the molecules they bind. Moreover, the repertoire of molecules they bind will probably depend in part on the precise structures of their GAG chains, structures which defy easy analysis. Nevertheless, PGs are likely to continue to receive increasing attention in neurobiology, as their *in vivo* distributions and *in vitro* activities suggest that they are widely involved in nervous system development and function.

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The Cat-301 immunoreactive CSPG of brain is shown to have properties similar to those of aggrecan. Also, authentic aggrecan from cartilage is shown to react with Cat-301, as well as to another monoclonal antibody that recognizes the same brain PG as Cat-301. Nevertheless, the Cat-301 antigen can be distinguished from aggrecan by virtue of containing few, if any, KS chains and having a lower buoyant density (indicative of a lower carbohydrate/protein ratio) than aggrecan. The data suggest that Cat-301 is either a modified form of aggrecan, or a new member of the aggrecan family.

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