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Review

Cell biology of astrocyte proteoglycans

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Abstract. A number of studies have demonstrated that the nervous system produces multiple molecular species of proteoglycans (PGs), and that these PGs on cell surfaces and in the extracellular matrix of the nervous system have the potential to act both as ligands for and receptors on neurons. PGs are now known to play an important role in cell-cell and cell-substrate interactions. This review focuses on a discussion of the expression, characterization and modulation of individual astrocytic PGs and the role that astrocytic PGs play in both the development and pathophysiology of the nervous system.

Key words. Proteoglycan; heparan sulphate; chondroitin sulphate; glycosaminoglycan; astrocyte.

Introduction

Growth responses in the central nervous system (CNS) are determined and regulated by the interaction of neurons with extraneuronal molecular species. Neuronal growth modulatory molecules may be grouped into two general classes – diffusible neurotrophic factors (neurotrophins) and nondiffusible molecules, which include extracellular matrix (ECM) ligands and cell adhesion molecules (CAMs). Proteoglycans (PGs) are a family of proteins bearing sulphated glycosaminoglycans (GAGs), unbranched polysaccharide chains with characteristic repeating disaccharide units that are found on the surface of cells, within basement membranes and in the ECM. Biochemical evidence has confirmed the presence of a number of molecular species of each family of PG in the nervous system [1–3]. These PGs have been implicated in a wide variety of cell functions such as neuronal survival, adhesion, determination and migration, axonal growth and guidance, synapse formation and glial differentiation during the development of the nervous system. The functional roles of PGs in the nervous system have been the subject of several recent reviews [2–4]. This review will therefore focus on recent advances demonstrating the expression, characterization and functional roles of astrocytic PGs in the regulation of neural development, neurite outgrowth, and morphogenesis.

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General aspects of PGs

PGs are major glycoconjugates, located on the cell surface and in extracellular spaces, consisting of a core protein with one or more GAG side chains covalently linked. The diversity of PGs is dependent upon differential expression of genes encoding core proteins as well as variation in the number, length and types of GAG chains. GAGs are large carbohydrate polymers with repeating disaccharide backbones consisting of a hexosamine and hexuronic acid. GAGs bind to many other molecules through charge interactions. The PGs can be considerably larger than their core proteins because of the large size of the GAG chains. In addition

to their GAG chains, both N- and O-linked oligosaccharides may be covalently attached to PGs. Synthesis of the repeating polymers that are characteristic of the GAG chain of each family of PG is carried out by the concerted action of specific transferases followed by modification of the polysaccharides. Biosynthesis of PGs involves translation of a protein template followed by a series of posttranslational modifications, which include elongation of GAG chains by glycosyltransferase activity followed by sulphation of GAGs by sulphotransferases [5]. Sulphation is a critical determinant of GAG charge density, which may be essential to the molecular interactions of the GAG with the neuronal cell surface or extracellular matrix. These processes are responsible for the vast heterogeneity of structure and function of PGs. Thus, in addition to core protein synthesis, both enzyme activity (glycosyltransferases, sulphotransferases) and substrate availability (sugar nucleotides, the sulphate donor phosphoadenosine 5-phosphosulphate) are important in the regulation of PG synthesis.

To date, four major families of PGs have been identified – heparan sulphate (HS) and heparin, chondroitin sulphate (CS) and dermatan sulphate (DS), keratan sulphate (KS) and hyaluronic acid (HA). The basic structures of the GAGs of these PGs have been summarized elsewhere [1–4]. The major species of GAGs identified in the CNS are CSPG and HSPG, which include the ECM CSPGs of the aggregan family such as Cat-301, transmembrane PGs such as NG2 CSPG and the syndecan family of HSPGs, and glycosylphosphatidylinositol (GPI)-linked HSPGs [6–9]. CS is the most abundant sulphated GAG in the brain. Most CSPG families are constituents of the ECM. HS has been shown to have more structural diversity than other GAGs. Most HSPGs are associated with the plasma membrane in three ways: direct intercalation of the core protein into the lipid bilayer, intercalation through a GPI anchor covalently bound to the core protein, and specific or relatively nonspecific interactions between HS chains and other molecules associated with the plasma membrane [10]. HA is a polymerized extracellular matrix GAG and has the simplest GAG structure, containing a disaccharide repeating unit composed of glucuronic acid linked to *N*-acetylglucosamine. HA exists as a free GAG without sulphation, not covalently conjugated to core proteins. HA synthesis takes place in a compartment associated with the cell surface. A number of molecules can bind to HA via specific, noncovalent interactions which extend over defined numbers of the disaccharide repeat units. Some HSPGs or KSPGs are found to be anchored to the plasma membrane by GPI (cerebroglycan, glypican and OCI-5) [11–13].

Recent advances in molecular cloning and sequencing analysis have identified diverse types of PG core proteins, which share similar sequence motifs and can be grouped into several families. The core proteins are also critical factors in determining the diversity of PGs. The growth modulatory activities of PGs on cell surfaces and in the extracellular matrix have been documented. Because of their multivalency, PGs have the potential to act both as ligands for and as receptors on neurons [14]. PGs interact with at least three classes of neuronal cell surface receptors that have been implicated in neuronal growth and adhesion – CAMs, integrins and a cell surface galactosyltransferase [15–17]. PGs also interact with extraneuronal matrix proteins and many growth factors involved in neurite growth such as fibroblast growth factor (FGF), laminin, fibronectin and cytotactin [16], thus acting as ligands. In addition, GAGs in the extracellular milieu mediate other ligand-receptor interactions by presenting growth modulatory molecules to neuronal receptors [18]. Such interactions may play important roles in many biological processes that regulate cellular proliferation and differentiation. The functional interactions between PGs and molecular species on the neuronal surface are mediated by the GAG chains, and the degree of sulphation of the GAG is an important determinate of the neurite-promoting activity of the PGs [14, 19]. Thus, functional interactions involving PGs are subject to regulatory events that operate at the level of posttranslational modification of the core protein.

Expression and physiological regulation of PGs in astrocytes

The nervous system is composed of two major cell types, neurons and neuroglia. The neuroglia family is comprised of astrocytes, oligodendrocytes and microglia with different morphological phenotypes and functions of each cell type. Astrocytes, the major glial population in the nervous system, are characterized by their expression of glial fibrillary acidic protein (GFAP) [20]. Recent studies have demonstrated that glial cells are also among the most functionally diverse group of cells in the nervous system in early development and in the adult [21, 22]. It is becoming apparent that astrocytes are intimately associated with neurons within the CNS and interact with neurons via complex molecular signals such as diffusible peptide factors, membrane-associated macromolecules and extracellular adhesion factors. Astrocytes are now known to synthesize and release multiple molecules, including peptide growth factors (e.g. nerve growth factor, FGF, ciliary neurotrophic factor), neurotransmitters (e.g. glutamate, norepinephrine, neuropeptide Y), CAMs (e.g. N-CAM, L1, N-CAD) and ECM proteins (e.g. laminin, fibronectin), thus functioning independently or cooperatively with

Table 1. Proteoglycans expressed in astrocytes.

PGs	Major GAG	Core protein (kDA)	Expression	Method	Reference
Hylauronan-binding PGs					
Brevican	CS	145	cultured astrocytes	Northern blot	[7, 44, 47]
				immunohistochemistry	
			released by astrocytes	in situ hybridization	
Neurocan	CS	245	cultured astrocytes	immunohistochemistry	[7]
			released by astrocytes	immunoblotting	
Transmembrane PGs					
NG2	CS	300	cultured type 2 astrocytes	immunohistochemistry	[56–58]
			O2A progenitor cells		
			smooth protoplasmic astrocytes	in situ hybridization	
RPTPβ	CS	310/300	cultured type 1 astrocytes	Northern blot	[75, 154]
			O2A progenitor cells		
			C6 glioma cells		
Soluble PGs					
Appican	CS	140–250	cultured astrocytes	Western blot	[61, 62, 64, 66]
			released by astrocytes	Western blot	
			C6 glioma cells	Western blot	
APLP2	CS	110, 100	C6 glioma cells	Western blot	[65, 69]
			astrocytes	immunohistochemistry	
Phosphacan	CS, KS	400, 360	O2A progenitor cells	Northern blot	[73, 75–77, 32]
			astroglia	immunohistochemistry	
			synthesized by radial glia	in situ hybridization	
Astrochondrin	CS	380, 360, 260	astrocytes	immunohistochemistry	[78,79]
Leucine-rich PGs					
Biglycan	CS/DS	43	cultured astrocytes	Northern blot	[81]
			released by astrocytes	in situ hybridization	
Decorin	CS	~40	astrocytes	immunohistochemistry	[86]
			reactive astrocytes	immunohistochemistry	
HSPGs					
			cultured astrocytes	HPLC	[28, 29, 90]
			released by astrocytes	HPLC	
			astrocytic surface of neonatalcortex	immunofluorescence	

neurons [23–30]. PGs are also among these astrocyte-derived neuroactive molecules.

PGs are widely expressed throughout the CNS and primarily localized in two major areas: associated with the plasma membranes or within basement membranes [1–3, 31]. Immunological and molecular biological studies have revealed the presence of a specific PG in multiple cell types, multiple species of PGs in a single type of cell and cell or tissue-specific PG synthesis [32, 33]. Although neuronal PGs are considered to be the main cellular source of PGs in the brain, a crucial role for astrocyte PGs in the regulation of cellular differentiation, neurite outgrowth and synaptogenesis has been determined. HS, CS and DSPGs all are produced by cultured mammalian glial cells and malignant glioma cells [34], and human glial cells also synthesize and release both CSPG and hyaluronic acid [35]. Using immunocytochemical and biochemical techniques, HSPGs and CSPGs have been shown to be localized on the astrocytic cell surface and are regulated during development [29, 36–38]. Immunoblot and immunocytochemical analyses have demonstrated that cerebral cortical astrocytes express high levels of CS-6-PG [39]. In addition, CSPGs as detected by the antibody CS-56, which reacts specifically with CS-4- and CS-6-sulphate, are found to be expressed in astrocyte-Schwann cell cocultures [40]. In addition, HA has been shown to be synthesized in and released from cultured astrocytes and glioma cells but not in neuronal cell lines including Neuro 2a neuroblastoma cells and PC12 cells [33, 41–43]. To date, many astrocyte PGs have been identified in the nervous system (table 1). The PGs contributed by astrocytes to the extracellular milieu provide the appropriate substrates for neuron adhesion, process extension and other cell-cell interactions. Expression of these PGs in vivo and in vitro is regulated during development and under certain physiological and pathological features such as nerve injury and regeneration, and Alzheimer's disease.

Table 2. Possible functions of astrocytic proteoglycans.

PGs	Functions	References
NG2	binds to type VI collagen interacts with and modulate platelet-derived growth factor receptor-α inhibits neurite outgrowth of granular neurons inhibits cell migration	[54, 149, 151]
Biglycan	supports cultured neuron survival	[81]
Decorin	binds to TGF-β family	[85]
DSD-1	promotes neurite outgrowth of hippocampal and mesencephic neurons	[97]
Brevican	inhibits neurite growth of granular neurons binds to tenascin-R	[47]
Phosphacan	inhibits neuronal and glial adhesion and neurite growth binds to Ng-CAM/L1, NCAM and tenascin-C promotes neurite outgrowth of cortical neurons	[72, 152]
6B4	promotes neurite extension of cortical neurons promotes dendrite development promote tyrosine phosphorylation	[76]
Neurocan	inhibits neuronal adhesion and neurite growth binds to Ng-CAM/L1 and N-CAM binds to tenascin-C inhibits cell migration	(51, 153)
Astrochondrin	promotes granular cell migration mediates astrocytic processes extension binds to laminin and collagen type IV interacts with collagen III, V, I, II and IX	[78,79]
Appican	promotes neural cell adhesion to ECM modulates glial cell morphology	[67]

CSPGs

CSPGs are the most prominent PGs in the CNS [2]. Biochemical studies have demonstrated the expression of CSPGs both in neurons and astrocytes in vivo [36], and in vitro [38] during CNS development. The extracellular labelling of CS is detected in granular and molecular cell layers of neonatal rat cerebellum at early developmental stages, and intracellular labelling of astrocytes appears at late stages [36]. So far a number of individual CSPGs have been found to be expressed on and associated with astrocytes, the cell surface of which may probably mediate important neuronal-glial interactions (table 2).

Brevican. Brevican belongs to the aggrecan/versican/neurocan family and was first isolated and characterized from bovine brain [44]. Like other members of this family, brevican contains a HA-binding domain in its N-terminal region, an epidermal growth factor-like repeat, a lectinlike domain and a complement regulatory proteinlike domain in its C-terminal region. Bovine brevican presents in two forms: a 145-kDa full-length form and an 80-kDa N-terminally truncated form. The main difference between brevican and other members of its family is that the central regions of brevican have a much shorter central GAG attachment region. Since only a portion of the molecule is substituted with GAG, the rest are free protein molecules. As there are a number of brevican molecules devoid of GAG chains present in the brain, brevican is considered a part-time PG acting both as PG and as free protein [44]. Cloning of the complete coding sequence of members of the aggrecan/versican family in the rat brain has identified and characterized two isoforms of PG core proteins [45]. One form has 82% sequence homology with bovine brain brevican. Both proteins appear brain-specific and act as part-time PGs. Another isoform is likely to be attached to the cell membrane via a GPI anchor. A recent study has also identified a putative brain-specific hyaluronan-binding protein (BEHAB) as a partial brevican complementary DNA (cDNA) [46]. Brevican expression in the brain using northern blot indicates that a single 3.3-kb transcript is predominantly expressed in astrocyte cultures but not in primary neuronal cultures, demonstrating that astrocytes are the main cellular origin of brain brevican [44]. Immunoelectron microscopic studies have shown that cultured astrocytes express brevican on their cell surface. Brevican is also synthesized by astrocytes as determined by in situ hybridization. When added to culture medium, brevican has an inhibitory effect on neurite outgrowth from

granular neurons [47]. While two brain HSPGs, named glypican and cerebroglycan, have been reported to be GPI-anchored PGs, the isoform of rat brevican may be the only GPI-anchored CSPG expressed in the CNS [45].

Neurocan. Neurocan, isolated from rat brain by immunoaffinity chromatography using the 1D1 monoclonal antibody, is a developmentally regulated CSPG specifically expressed in the nervous system [7, 32, 48]. Neurocan is a member of the aggrecan/versican family of HA-binding CSPGs [49]. The deduced amino acid sequence reveals a core protein containing a 22-amino acid signal peptide sequence followed by an immunoglobulin domain and tandem repeats characteristic of the HA-binding region of aggregating PG, and an RGDS sequence for integrin binding [48]. Neurocan consists of a major core glycoprotein of MW 245 kDa in the early postnatal rat brain and an average of three 22-kDa CS GAG chains. The C-terminal region of neurocan has 60% homology to the C-terminal of fibroblast and cartilage PGs, versican and aggrecan, including two epidermal growth factor-like domains, a lectinlike domain and a complement regulatory protein-like sequence. The central domain of neurocan has no homology with other reported protein sequences [48]. Neurocan is developmentally regulated, with a rapid increase in concentrations during the late embryonic and early postnatal period, reaching a peak at approximately postnatal day 4, then declining to below embryonic levels in adult brain [50]. The N-terminal fragment of neurocan can bind to HA and undergo extensive proteolytic processing during brain development. The presence of a lectinlike domain in the C-terminus together with an N-terminal HA-binding region could allow neurocan to serve as a connection between the cell surface and extracellular HA [48]. In addition, neurocan can bind to tenascin but not to laminin and fibronectin through its core protein [51]. Immunoblotting study using 1G2 monoclonal antibody has demonstrated that neurocan is detected in conditioned medium not only of highly enriched cultures of fetal rat cortical neurons but also of pure cultures of mature astrocytes and astrocyte-conditioned medium. Cultured astrocytes release neurocan into culture medium at levels 12- to 20-fold higher than cultured neurons [7].

NG2 proteoglycan. NG2 is a large transmembrane CSPG of molecular weight (MW) 400–800 kDa containing a core protein of MW 300 kDa and two or three CS chains, originally identified in rat neural cell lines [52, 53]. The predicted primary structure is an integral membrane protein with a large extracellular domain (2224 amino acids), a single transmembrane domain (25 amino acids) and a short cytoplasmic tail (76 amino acids). The extracellular region can be divided into three domains: an amino terminal cysteine-containing domain which is stabilized by intrachain disulphide bonds, a serine-glycine-containing domain to which CS chains are attached and another cysteine-containing domain. There are internal repeats, each consisting of 200 amino acids, found in the extracellular domain of NG2. These repeats contain a short sequence that resembles the putative Ca^{++}-binding region of the cadherin family [54]. Using a solid-phase binding assay, the NG2 PG has been shown to bind to collagens V and VI through the central nonglobular domain of its core protein [54, 55]. The NG2 PG is considered to be an integral membrane PG, thus suggesting its role as a cell surface receptor. In vivo and in vitro the NG2 PG has been found on the cell surface of a particular subpopulation of glial cells, a class of smooth protoplasmic astrocytes which have a stellale morphology found mainly in gray matter [56]. In dissociated cultures of postnatal rat optic nerve and cerebellum, this PG is also expressed on a population of cells which are believed to be type 2 astrocytes. These NG2-expressing cells display the phenotypic plasticity that is characteristic of OA2 progenitor cells. In serum-containing conditions, type 2 astrocytes continue to express NG2 for up to 10 days in culture, while mature oligodendrocytes and astrocytes do not express NG2 [57, 58]. Using immunohistochemical staining, these unusual NG2-bearing cells have been shown to have the potential to respond to brain injury by increasing the expression of NG2 [59]. The NG2-positive cells adjacent to the lesion sites display a transient increase in immunoreactivity to NG2 antibody compared with cells in undamaged areas. The increased NG2 immunoreactivity is accompanied by an increase in messenger RNA (mRNA) levels of NG2 detected by in situ hybridization [59].

Appican. The amyloid precursor proteins (APPs) are ubiquitously present in all mammalian tissues, including the nervous system, and at least three APP protein isoforms have been identified, encoding protein 695, 751 and 770 [60]. Recently, a significant fraction of APP, named appican, has been identified to exist as the core protein of a CSPG, with MW ranging from 140 kDa to 250 kDa released by the glial cell line C6 [61]. Further studies have confirmed that the cell surface of C6 glial cells expresses both full-length and truncated forms of CS APP [62]. The core protein of appican is a spliced isoform of APP lacking exon 15, which results in the joining of exons 14 and 16, and the formation of a consensus sequence for CS chain attachment to the serine residue (fig 1) [63]. Cell-associated appican contains full-length L-APP, while soluble appican contains a truncated form of APP which lacks transmembrane and cytoplasmic domains. Appican is expressed in purified PG preparations of human and rat brain. Both secreted and cell-associated appican are detected in astrocyte cultures but not in neuron, oligodendrocyte or

microglial cultures [64]. The appican expression in culture appears to depend on the cell type and culture condition, as the number and length of CS GAG chains are modified in different culture conditions [64, 65]. Western blotting using the monoclonal antibody 22C11 against APP has shown that large amounts of CS-type APP are secreted into cultured medium by astrocytes obtained from fetal rat brains, while only a small amount of CS-type APP is detected in a soluble preparation of PG from 10-day-old rat brain [66]. While the biological function of appican is still unclear, a recent study has shown that this PG promotes neural cell adhesion to ECM and has a role in modulation of glial cell morphology [67].

Amyloid precursor-like protein 2 (APLP2) belongs to a family with homologies to the APP protein. Recent studies have shown that APLP2 is expressed as the core protein of CSPG in stably transfected Chinese hamster ovary and transiently transfected African green monkey kidney cells [68]. While APLP2 is highly homogeneous with APP, APLP2 does not contain the Aβ sequence. Using specific antisera, the endogenous expression of appican is found in two neural cell lines, C6 glioma and Neuro-2a neuroblastoma, while the secreted APLP2 CSPGs with core proteins of 100 and 110 kDa are expressed more extensively not only in C6 glial cells but also in other nontransfected cell lines [65]. Immunocytochemical labelling with APLP2-specific antibodies indicates APLP2 immunoreactivity in cytoplasmic compartments in both neurons and astrocytes overlapping the distribution of the APP [69].

Phosphacan. Phosphacan, produced mainly by glial cells in the CNS, is identified and characterized from rat brain using the monoclonal antibody 3F8 [32]. Phosphacan has a MW of 500 kDa and a 400-kDa core protein in 7-day rat brain. The PG contains an average of four CS GAG chains, each approximately 28 kDa. A chondroitin/keratan sulphate PG hybrid (phosphacan-KS) recognized by a monoclonal antibody 3H1 to the KS is also present in rat brain with a size of 500 kDa containing three to five chondroitin 4-sulphate chains and three to six KS chains in the early postnatal rat brain [32]. The primary structure of phosphacan reveals that its core protein is a possible mRNA splice variant of the extracellular domain of a receptor-type protein tyrosine phosphatase (RPTP) z/β family, which acts as a signal transduction molecule involved in cell-cell and cell-matrix interactions [70, 71]. Phosphacan, like neurocan, binds to tenascin and to the neuron-glia cell adhesion molecules Ng-CAM/L1, N-CAM and TAG-1/axonin-1 with high affinity via its core protein [56, 72].

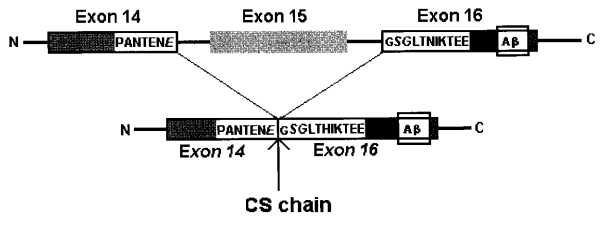

Figure 1. Diagrammatic structure of the APP region for the formation of the consensus sequence EXSG for the attachment of a CS chain. This site is 16 residues upstream of the Aβ sequence of APP. Adapted from Pangalos et al. [63].

Immunocytochemical and in situ hybridization studies demonstrate that phosphacan is synthesized by astroglia, especially cerebellar Golgi epithelial cells [32, 73]. The sulphation, carbohydrate composition, oligosaccharide structure and localization of phosphacan in the nervous system is developmentally regulated. The expression of phosphacan in glial cells is increased dramatically during embryonic development [74, 75]. Using northern blot analysis, a 8.4-kb transcript of RPTP-β corresponding to phosphacan has been found to be the most abundant message during postnatal development and is expressed at high levels in glial cell cultures and O2A progenitor cultures [75]. Recently, a brain-specific CSPG, 6B4 PG/phosphacan, has been isolated from rat brain with a 300-kDa core protein substituted with CS and KS chains and HNK-1 carbohydrates. The 6B4 PG is found to be associated with radial glial fibers and may be synthesized by radial glia in addition to neurons [76, 77].

Astrochondrin. Astrochondrin, which carries the L2/HNK-1 and L5 carbohydrate structure, was first characterized from the murine CNS using the monoclonal L5 antibody [78, 79]. This neural CSPG is expressed by astrocytes in vitro, and digestion of the [^{35}S]methionine-labelled L5 PG from cultured astrocytes with proteinase-free chondroitinase indicates three major core proteins with apparent MWs of approximately 380, 360 and 260 kDa. The three core proteins of different size are similar in their proteolytic peptide maps. Astrochondrin is developmentally regulated with peak expression at postnatal day 8. Immunocytochemical localization of astrochondrin in the cerebellar cortex of 6-day-old mice reveals an association with the cell surface of astrocytes, including Bergmann glial processes and astrocytes in the internal granular layer or white matter. Astrochondrin interacts with collagen types III and V, and less strongly with collagen types I, II and IX, and not with collagen type VI. The interactions of astrochondrin with collagen types III and V is saturable and susceptible to increasing ionic strength, and can be competed by CS, heparin, and dextran sulphate, but not by HA, glucose-6-phosphate, or neuraminic acid [79].

Biglycan and decorin. Biglycan and decorin are two small chondroitin/dermatan sulphate PGs with a core protein of about 40 kDa that belongs to a growing family of proteins harboring leucine-rich repeats. The biglycan protein sequence contains 368 residues of MW 42 kDa for the complete sequence and 37 kDa for the secreted form. There are four discrete domains, one of which contains two sites of attachment of DS [80]. The deduced biglycan protein core is highly conserved across species. Astrocytes synthesize and release biglycan, which is found to support survival of neocortical neurons in vitro [81]. Northern blot analysis of total RNA prepared from highly enriched astrocyte cultures has revealed a single 2.9-kb biglycan transcript, and in situ hybridization has demonstrated that essentially all cells in these cultures express biglycan mRNA [81].

The core protein of decorin contains three Ser-Gly dipeptide sequences, to one of which is attached a single CS or DS chain [82]. Decorin core protein binds to fibronectin, thrombospondin and transforming growth factor-β (TGF-β) [83–85]. Binding of decorin to TGF-β inhibits TGF-β activity, which in turn can regulate decorin synthesis [85]. Immunohistochemical analysis has revealed the expression of decorin core proteins in adult rat astrocytes [86]. Decorin expression is also upregulated after lesions in the nervous system, and reactive astrocytes are believed to be the main origin of decorin in the brain after injury [86]. Following dorsal root crush the immunoreactivity of CS/DSPG, detected by 3B3 antibody labelling of core protein of CS-6-sulphated PG, is enhanced [86, 87].

HSPGs

HSPGs, less highly sulphated GAGs with similar structures to heparins, are heterogeneous with respect to the size of their core proteins and GAG structures [1, 88]. The structural diversity of HSPGs may suggest that HSPGs have diverse functions by interacting with multiple molecules which bind to GAG or core proteins. In the intact nervous system HSPGs account for a small fraction of PGs appearing in the soluble fraction of whole brain extracts, and the intact neuronal PGs released to conditioned medium comprise 25% of HSPGs [1, 89]. The nervous system also contains some HSPGs anchored to the plasma membrane by GPI [1], including glypican and cerebroglycan [11, 13]. HSPGs have been observed throughout the nervous system, are widely present in many cell types and are primarily associated with the plasma membrane or with the basement membrane.

Immunocytochemical observations have revealed the colocalization of HSPG and laminin on astrocytic surface of neonatal rat cortex in vitro [29]. Immunofluorescence staining has demonstrated the presence of anti-HSPG immunoreactivity on the cell surface of astrocytes from embryonic spinal cord cultures [90]. Quantitative biosynthetic studies with highly enriched astrocytic cultures have shown that astrocytes in vitro produce a family of PGs that are displayed on the cell surface and released to the extracellular environment [28]. Analysis of the GAGs of these PGs reveals the presence of HSPGs with a 1:1 ratio of HS to CS/DS in cell extracts and a 1:6 ratio in medium. All of the GAGs are associated with core proteins that are integral to the cell membrane and associated with the cell surface by noncovalent interactions involving GAGs. For HS, approximately 20% of the cell surface fraction is heparin releasable, while between 46 and 68% of the HA, CS and DS is associated with the cell surface by noncovalent interactions mediated by GAGs. HS released to medium is undersulphated relative to that associated with cells [28]. Consistent with earlier observations [1], HSPGs contribute only 15–20% of the total sulphated pool of PGs released by astrocytes. HSPGs have been detected in the aged human brain by immunocytochemical staining [91]. Recent biochemical isolation of PGs from different sectors of embryonic mouse midbrain have demonstrated that HSPGs are compartmentally present in both lateral and medial midbrain astroglial cultures [92]. While the regulation of HSPGs during development is not clear, studies by Chisamore et al. have demonstrated that glutamate activation of excitatory amino acid receptors induces HSPG release from and synthesis by fetal hippocampal astrocytes mediated via kainate and metabotropic receptors [93].

Functional roles of astrocyte PGs

Modulation of neuronal survival, neurite growth and differentiation

The functional significance of PGs produced by astrocytes has yet to be fully elucidated. Astrocytes and their released trophic factors are believed to mediate neuronal survival, differentiation and regeneration by providing multiple molecular signals including PGs. Some astrocytic PGs have been shown to promote survival and neurite growth during development and following lesions. In earlier immunocytochemical studies, both HSPG and laminin have been shown to be expressed on the surface of cultured astrocytes and regulated with morphological differentiation. Neurites growing on astrocyte monolayers contact areas where complexes of HSPGs and laminin are deposited [29]. Observations using cell culture systems suggest that embryonic neurons and type 1 astrocytes in vitro synthesize both HSPGs and CSPGs and release those PGs into conditioned medium. HSPGs promote neurite growth by complexing with the glycoprotein laminin

and the neuronal cell surface by noncovalent interactions between the HS GAGs of the PG [94]. PGs produced and released by primary astrocytes in vitro have been characterized by their growth-promoting activities. On a poly-D-lysine substrate, the rank ordering of specific neurite growth-promoting activities is 330 kDa HSPG > 100 kDa HSPG/CSPG mixture or hybrid > 330 kDa CSPG > 50 kDa CSPG/DSPG mixture or hybrid and the 31-kDa sulphoprotein [95]. Consistent with the suggestion that some CSPGs are found to have neurite growth-promoting effects rather than inhibitory effects [96], a neural CSPG, designated as DSD-1-PG identified by monoclonal antibody 473HD and produced mainly by astrocytes, has been shown to promote neurite growth from postnatal 14-day mesencephalic and postnatal 18-day rat hippocampal neurons [97]. The CSPG astrochondrin is involved in cerebellar granular cell migration and mediates extension of astrocytic processes on collagen and laminin, but not on fibronectin [78, 79]. A small CS/DS PG, biglycan, is synthesized and released from cultured astrocytes and shown to support neuronal survival of neocortical neurons in vitro [81].

CSPGs are well known to inhibit neurite growth in vitro [98]. Several CS, CS/DS or KS PGs are expressed in astrocytes from different regions of brain and comprise astroglial barriers to axon outgrowth, resulting in concentration-dependent inhibitory effects on neurite growth. These PGs can interact with other neurite growth-modulating molecules such as laminin, and the inhibitory activity of PGs can be overcome only by the presence of large amounts of laminin in the cultures [99]. CSPGs produced by neonatal rat astrocytes in vitro, while able to support neurite growth when offered as the only substrate, can inhibit the growth stimulatory effects of HSPGs [100]. A molecular component associated with the extracellular matrix of astroglial scars following trauma has been identified as a CSPG, the expression of which correlates with the inability of the reactive astrocytes to support neurite growth [101].

The morphological isolation of two subpopulations from mixed astrocytic cultures together with immunocytochemistry and immunoblotting shows that a subpopulation of astrocytes, termed rocky astrocytes, characterized by a fibrous, uneven surface, express high levels of chondroitin-6-sulphate PG, which may play an important role in rocky astrocyte process growth [39]. Some astrocytic cell lines exhibit diverse effects on neurite growth primarily based on the ECM they produce [102, 103]. Permissive and nonpermissive astrocytic cell lines, Neu7 and A7, as well as primary cultured astrocytes produce one or more dermatan/keratan PGs in varying amounts which block the neurite growth-promoting effects of laminin, thus acting as either a poor or good promoter of axonal growth. The effects of PGs produced and released by Neu7, A7 and primary cultured astrocytes are significantly disrupted by treatment of those cells with the inhibitors of PG synthesis, sodium chlorate and β-D-xyloside [103]. The latter inhibits assembly of GAGs onto the protein core by acting as a competitive substrate for the galactosyltransferase required for the synthesis of linkage region [104], and has been shown to block HSPG-mediated neurite growth [89]. The PG synthesis inhibitors also influence axonal growth on three-dimensional primary astrocytic cultures, which closely mimics the in vivo effects of astrocytes on axon growth [103].

Modulation of synaptogenesis

Activation of the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor is required for experience-dependent modifications of the synaptic connections that occur in the developing nervous system [105, 106]. Neurotransmitters have been shown to play prominent roles as molecular signals influencing the structure of neurons during development [107]. While diffusible growth factors such as the neurotrophins appear to be regulated by synaptic activity and thus may play a role in mediating growth effects and modulating neural connections in the developing and adult nervous system [108], the alterations in adhesive interactions at synapses mediated via the ECM are also thought to be important for enduring activity-dependent synaptic modification [109, 110]. Information is now emerging to suggest a role for PGs in activity-dependent neurite growth. Visual deprivation during the critical period of development of central visual pathways inhibits the expression of the Cat-301 CSPG, and the expression of this PG may be regulated by NMDA receptor activity [111]. The ECM CSPGs neurocan and cytotactin-binding (CTB) PGs participate in the formation of barrel structure in the somatosensory cortex, and their expression and synthesis are also regulated by neuronal activity [3, 112]. More recently, glutamate activation of NMDA and metabotropic receptors has been shown to induce the synthesis and release of PGs with neurite growth-promoting activity from fetal hippocampal neurons and astrocytes in dissociated culture [93, 113]. The synthesized and released HSPGs regulated by excitatory amino acid receptor (EAA) activation are able to promote neurite formation from chick embryo dorsal root ganglia neurons [113] and upregulation of neuron-specific growth-associated genes and neurite extension [114, 115], providing evidence that neuronal PGs regulated by activation of EAA receptors mediate neuronal growth responses.

Neurons are intimately associated with astrocytes during early development, and glutamate released from neurons during neuronal activity is taken up by astro-

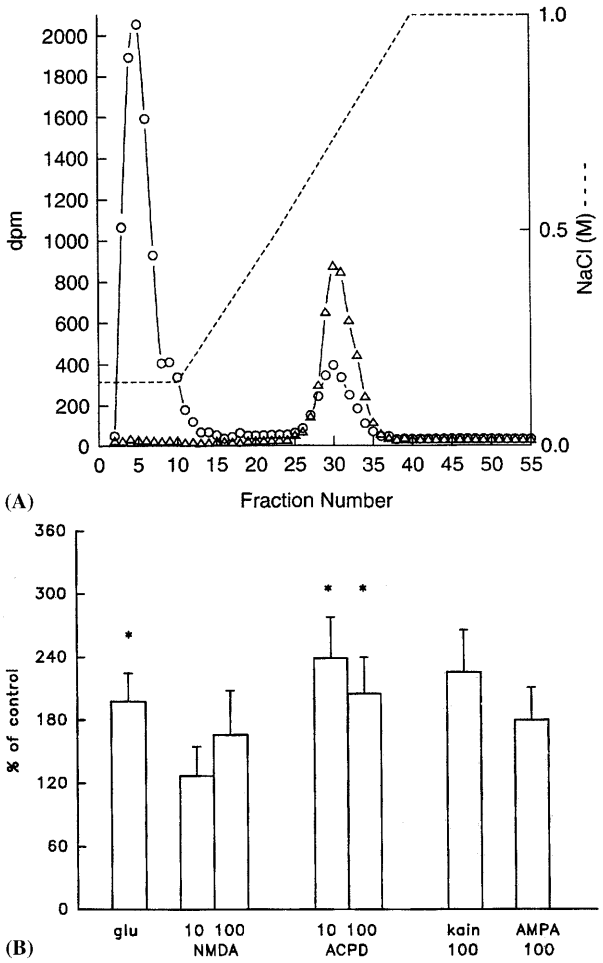

Figure 2. Excitatory amino acid regulation of astrocyte proteoglycans. (*A*) A representative chromatographic run of PGs released during a 5-min exposure to control conditioning solution following radiolabelling of cultures for 24 h. ○, ^{3}H-leucine; △, ^{35}S-O_4. (*B*) Excitatory amino acid receptor activation of PG release from astrocytes. Glutamate (glu), NMDA, the non-NMDA agonists kainic acid (kain) and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA), and the metabotrophic receptor agonist 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) were used at the concentrations shown (μM) during the 5-min exposure period. The data shown represent ^{35}S-O_4 incorporation into released PG and are expressed as percent of control PG release. * $p < 0.05$ vs. control. Reprinted with permission from: Chisamore B., Solc M. and Dow K. E. (1996) Excitatory amino acid regulation of astrocyte proteoglycans. Dev. Brain Res. **97:** 22–28, © 1998 Elsevier Science, Amsterdam.

cytes and results in altered glial morphology and activity [116, 117]. Axonal activity influences the development of glia in neonatal mammalian optic nerves, and the regulation of this development may involve activity-dependent release of signalling molecules, all demonstrating that glia play a critical role in the modulation of synaptic efficacy [118, 119]. The synthesis and release of PGs from fetal hippocampal astrocytes have also been shown to be upregulated by glutamate activation of EAA receptors, and the effects are mediated via kainate and metabotropic receptor activation, with activation of NMDA alone having no effect (fig. 2) [93]. These results are in keeping with previous data demonstrating the presence of non-NMDA and metabotropic receptors and the absence of NMDA receptors on astrocytes [120, 121]. Activity-dependent regulation of astrocytic PGs may thus be involved in the spatial regulation of neuronal growth subserving connectivity during development and adaptive responses of the mature nervous system.

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of senile plaques (SP) and neurofibrillary tangles (NFTs) within certain brain regions [122–125]. Loss of synapses indicative of a neuropathy leading to cell death reflects a degenerative phenomenon and is an early marker of AD severity. Dystrophic neurite formation is an abortive regenerative response of neurons associated with late AD, and contributes to disease severity because it leads to abnormal synaptic connectivity and/or neurotransmitter function. SPs are composed principally of a core of amyloid β-protein (Aβ), a 39–43-amino acid peptide, proteolytically derived through APPs, which has been shown to be a CSPG core protein and associated with the ECM. Widespread APP, while present, is considered as necessary but by itself insufficient for the development of AD dementia [126, 127]. While both SPs and NFTs are the neuropathological hallmarks of the disease, the relationship between these findings in the AD brain and the severity of clinical dementia has not been established with certainty.

It is now well established that PGs are a feature of SPs in AD and are also associated with NFTs [128]. APPs are widely distributed in many mammalian tissues, with the highest expression in the brain, and have been shown to play an important role in the pathogenesis of AD. There is evidence that the neurite growth-promoting activity of APP is involved in the interactions between APP and specific PGs. The interaction of specific APP-binding PGs with amyloid plaques may disturb the normal function of APP and contribute to the neurite degeneration [129]. APP has been shown to bind to HSPG, HS GAG and heparin both in the ECM and on the cell surface [130–132], which potentiates the effect of APP on neurite growth. Three distinct classes of PGs/GAGs (HSPGs, CSPGs and DSPG) have been immunohistochemically localized both in SPs and NFTs [129, 133]. HSPG is the most prominent PG detected in SPs in the AD brain. Highly sulphated PGs are also detected in dystrophic neurite (DNs) in the regions of

SPs. Perlecan is a major and specific HSPG present within the Aβ deposit in AD brain [4, 91, 134]. Decorin, a small CS/DS PG, is also found in the periphery of SPs and within NFTs of the AD brain [135]. Immunohistochemical studies have further demonstrated the presence of three CSPGs of varying degrees of sulphation in the dystrophic neurites of SPs and within NFTs [133]. More recently, KS PGs have been immunolocalized to the dystrophic neurites of the neuritic plaques of AD and within synapses in the AD brain [136]. Thus there are at least four different classes of PGs present within the neurite plaques of AD, demonstrating that PGs are constituents of those structural features of AD that correlate best with disease severity DNs and NFTs. The appearance of PGs associated with SPs and NFTs may speak to the neuronal or astrocytic origins of the PGs found in AD. While neurons constitutively synthesize and release HSPGs and CSPGs, and during regenerative growth in vivo both PGs are transported by growing axons [137], astrocytes are also possible cellular sources of PGs involved in the interactions in AD. Both HSPGs and CSPGs are reported to be associated with astrocytes, SPs and NFTs. In the AD brain, HSPGs have been identified and localized to accumulate in the amyloid deposits of SPs and congophilic angiopathy by two immunocytochemical probes. Both astrocytes and neurons may be involved in HSPG deposition in these lesions [138]. HS-like molecules are present in astrocytes, neurons, SPs and NFTs and colocalize with the Aβ protein in the AD brain [91]. C6 glioma cells express full-length APP CSPG. This cell line and primary cultured astrocytes can also release the CSPG form of APP into culture medium [61, 62, 66]. Recent studies have shown that appican is the CS PG form of APP, suggesting a role for this PG in AD [61]. The secretion of appican has been shown to depend on both cell type and growth conditions, and this PG is produced mainly by primary cultured astrocytes and C6 glioma cells as well as N2a neuroblastoma cells, but not by neurons. The regulation of appican expression is independent of APP synthesis [64, 65]. CSPG-modified forms of APLP2 are also expressed in a subset of neuritic plaques in the AD brain and are particularly conspicuous in large dystrophic neurites [69]. Astrocytes have been shown to guide neuronal migration and inhibit neurite regeneration following neuronal injury [21]. In the AD brain, they have been shown to surround neurite plaques. Thus, the specific expression of astrocytic appican may suggest a role of this PG in neurite growth or regeneration in AD.

Aβ protein is the major component of the extracellular amyloid deposits in the AD brain. Increased production of Aβ is believed to play an important role in the development of AD. Recent observations have shown that PGs can significantly affect the rate of removal of Aβ [139, 140]. While astrocytes are not able to process the Aβ deposit, these cells release glycosaminoglycase-sensitive molecules that inhibit the subsequent removal of Aβ by microglia. Addition of purified CSPG to Aβ that is in medium or focally deposited results in significant inhibition of peptide removal by microglia [139]. Astrocytic PGs have also been reported to be a potential mechanism for neuritic dystrophy in AD. Astrocytes surrounding SP cores can produce CSPGs. In the presence of astrocytes, neurons appear to avoid the astrocyte-conditioned SP cores, which may be due to the axonal growth inhibitory nature of CSPGs [141]. In cortical and hippocampal cultures, astrocytes are triggered into a functionally reactive state by immobilized Aβ, and thus synthesize and release PGs, which may account for the inhibitory effects on neurite outgrowth [142, 143]. Thus, PGs released by the interactions between astrocytes and SPs appear to facilitate decreased axon density and synaptic loss in the AD brain.

Brain injury and reactive astrocytes

Injured axons in the CNS fail to regenerate compared with injured peripheral axons. Regenerative responses in the CNS are determined, to a great extent, by molecular species of the extracellular milieu and their interactions with the growth cone of the neuron. One of the crucial determinants for the failure of regeneration lies in the formation of a glial scar formed by reactive astrocytes, which has been implicated as a barrier to axonal regeneration. While astrocytes may possess both axon growth-promoting and axon growth-inhibitory activities, recent studies have started to establish that some type of astrocytes in injured lesions can synthesize and release multiple cell surface or ECM molecules providing inhibitory substrates for axonal regeneration following brain injury, thus contributing to the poor regenerative capacity of the CNS. The inhibitory effects of these molecules on neurite outgrowth have been associated with the expression of PGs [59, 101, 144–146]. Astrocyte-conditioned medium contains three PGs with neurite growth-modulating activity [28, 95, 102, 147]. Immunohistochemical studies have shown chondroitin-6-sulphate PG to be present in glial fibrillary acidic protein (GFAP)-positive astrocytes and around the scar in vivo. The expression of this CSPG is correlated with inhibition of neurite outgrowth in vitro [101]. Further studies indicate the presence of more than one species of PG or a single PG with both CS and HS chains. The major components of injury-enhanced PGs found in gliotic tissue are CSPGs with core proteins between 180 and 400 kDa. These astrocytic CSPGs induced by brain injury are able to inhibit axonal growth as determined by biological assays. The studies have also suggested that the inhibitory influence on

neurite outgrowth produced by injury-induced CSPGs is partially due to the interactions between CSPGs and growth-promoting molecules such as laminin, which is expressed by reactive astrocytes following injury [101, 148]. Recently, one of the identified CSPGs (NG2) that displayed inhibitory activity on neurite growth in vitro was found to be transiently upregulated in cerebellar astrocytes following a small puncture injury [59]. Together with the observations that NG2 inhibits neurite growth from cerebellar granule cells [149], these data suggest that increased expression of NG2 CSPG following brain injury may result in inhibitory effects on regeneration of damaged axons. In the transected postcommissural fornix of the adult rat, lesion-induced upregulation of CS/DS-PGs has been observed, while this CSPG, recognized by antibodies 3B3 and CS-56, is not shown to provide an impermeable boundary for regrowing axons [150]. Using immunocytochemical staining, two individual PGs, decorin and biglycan, have been shown to be upregulated in the transected postcommissural fornix of the adult rat [86]. At least one of them, decorin, is primarily expressed by astrocytes. Both decorin and biglycan have been shown to bind to TGF-β via their core protein, and to interact with fibronectin and thrombospondin, two strong promotors for neurite growth. It seems likely that upregulated CS/DS-PGs may be involved in the synthesis of these ECM molecules in injured tissues, and thus modulate the neurite outgrowth at lesion sites after injury.

Summary

The significant advances in the biology of PGs in the past years have suggested that astrocytic PGs play a critical role in the regulation of cellular differentiation, neurite outgrowth and synaptogenesis during the development of the nervous system. The various astrocytic PGs on the cell surface may also have important functions in some pathological conditions such as Alzheimer's disease and nerve injury and regeneration. Further work will prove the functional significance of PGs produced by astrocytes and define the functional interactions between astrocytic PGs and other molecular species on the cell surface.

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