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Proteoglycans and the Acute-Phase Response in Alzheimer's Disease Brain

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Abstract

Alzheimer's disease is a dementing disorder affecting increasingly large numbers of individuals in the aging population. The characteristic neuropathologic changes of Alzheimer's disease are the deposition of extracellular amyloid plaques, neurons containing neurofibrillary tangles, and neuronal cell loss. The A4 amyloid peptide is the major constituent of senile plaques. In addition to the A4 peptide, senile plaques contain a variety of molecular species, including proteoglycans and inflammatory components. The presence of proteoglycans in the amyloid deposits of Alzheimer's disease and of systemic amyloidoses suggests that these molecules play an active role in the pathogenesis of amyloidosis. However, the molecular mechanisms that lead to the codeposition of amyloid peptide with proteoglycans is still unknown. Recent evidence suggests that the metabolism of proteoglycans is altered in Alzheimer's disease patients. The acute-phase response observed in the brain of patients affected by Alzheimer's disease may be responsible for this effect. In this article, we discuss the role of proteoglycans in Alzheimer's disease, and the possible interactions between factors involved in brain inflammatory mechanisms and proteoglycans in the pathogenesis of Alzheimer's disease.

Index Entries: Glycosaminoglycans; amyloid; interleukin-1; nerve growth factor.

Abbreviations: AD, Alzheimer's disease; *βAPP*, β-amyloid precursor protein; CS, chondroitin sulfate; DS, dermatan sulfate; FGF fibroblast growth factor; GAG, glycosaminoglycan; GlcNAc, *N*-acetyl-glucosamine; $GlcNSO_3$, *N*-sulfate-glucosamine; GlcUA, glucuronic acid; HS, heparan sulfate; IdoUA, iduronic acid; IL, interleukin; KS, keratan sulfate; NGF, nerve growth factor; PG, proteoglycan; PNP, *p*-nitrophenylxyloside; snPG, secreted neuroblastoma proteoglycans.

Introduction

Cerebral amyloid deposition is one of the defining features and an early neuropathological lesion of Alzheimer's disease (AD). The main component of the amyloid deposits is a 40 amino acid peptide called A4 peptide that is derived from the β-amyloid precursor protein *(βAPP) (1–3)*. Within the *βAPP* molecule, the A4 peptide is located at the junction of the extracellular and transmembrane domains. *βAPP* can be proteolytically processed in two different pathways: a nonamyloidogenic and an amyloidogenic pathway. The nonamyloidogenic pathway results in the secretion of the extracellular domain of *βAPP* after an enzymatic cleavage of the molecule within the A4 peptide *(4,5)*. The amyloido-

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genic pathway, recently described by several authors (6–11), generates fragments containing the intact A4 peptide. The exact mechanism that results in the accumulation of the A4 peptide in the amyloid plaques is still unknown. The *βAPP* gene is located on chromosome 21. In Down's syndrome, the cerebral amyloid deposition appears to be caused by increased gene dosage leading to excessive amounts of *βAPP* (12). In AD brains, increases in *βAPP* gene expression have been also demonstrated via *βAPP* RNAs (13–15). From these observations, it is believed that amyloid deposition is a consequence of an excessive expression of *βAPP* and its subsequent amyloidogenic cleavage. The local brain inflammation described in the brain of AD patients (16–19) may be responsible for these effects. During the acute-phase response, several components, such as cytokines, growth factors, proteases, and protease inhibitors, are secreted. Interleukin-1 (IL-1), interleukin (IL-6), and nerve growth factor (NGF) have been shown to increase the synthesis of *βAPP* (20–25). The release of proteases and protease inhibitors may favor the amyloidogenic cleavage of *βAPP* (26).

Proteoglycans (PGs) are an another group of molecules that are believed to play an active role in AD (27). They have been shown to be present in the characteristic lesions of AD (amyloid deposits and neurofibrillary tangles) (28–31). PGs are also associated with other types of amyloidoses, both cerebral and systemic (32–36). Some of them are related to an inflammatory process, but as in AD, the role of the acute-phase response in the deposition of amyloid remains to be elucidated (37).

Proteoglycans in the Brain

PGs are a group of macromolecules composed of a protein core to which one or more glycosaminoglycan (GAG) chains are covalently linked. The GAG chains primarily consist of a linear repetition of disaccharide units containing *N*-acetylated hexosamine and either hexuronic acid or galactose units. These components carry sulfate substituents in various positions. Seven major GAGs have been identified: hyaluronic acid, which is nonsulfated and not linked to a protein core; chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate (DS), heparan sulfate (HS), heparin, and keratan sulfate. Although their structures are based on a repeating disaccharide unit, selective epimerization of the hexuronic acid and selective sulfation produces chains with specific properties. For PGs, in addition to the specificity of the GAG chains, specificity is also attributed to the core protein (38).

PGs are ubiquitous constituents of mammalian cell surfaces and extracellular matrices, including the specialized basal laminae that surround neural tissue (38). All the primary GAGs, except heparin, have been found in the mammalian central nervous system (39,40). Biochemical and immunochemical studies of PGs in human, rat, mouse, and embryonic chick brains have shown a large variety of PGs, demonstrating the abundance and structural diversity of these molecules in the central nervous system. The most abundant and well characterized are chondroitin sulfate proteoglycans (CSPGs) (40–45), which are present almost exclusively as the 4-sulfate isomer (46), and to a lesser extent, heparan sulfate proteoglycans (HSPGs) (40,47,48). In a recent study, 25 putative PG core proteins (derived from CSPGs and HSPGs) have been identified (49). Traces of dermatan sulfate proteoglycan (DSPG) and keratan sulfate proteoglycan (KSPG) have also been demonstrated (50,51), and a hybrid PG composed of CS and KS GAG chains has been characterized in the rat and embryonic chick brains (43,52). The primary structures of three PG core proteins have been recently determined by cloning techniques: NG2, a CSPG present in neural cells and astrocytes (53); neuroscan, a CSPG isolated from rat brain (54); and *N*-syndecan, an HSPG from rat Schwann cells (55).

There is evidence that PGs and GAGs are involved in a variety of neurobiological processes. A number of investigations have shown that distinct changes occur in the type and quantity of GAGs and PGs in the brain during development and/or aging (56–59). The developmentally regulated expression of PGs/GAGs within the mammalian brain suggests that these molecules are implicated in neuronal development and regeneration.

Studies of sulfated PGs and GAGs have shown a significant role of these molecules in influencing cell adhesion, neurite outgrowth, and neuronal polarity on specific substrata (laminin, fibronectin, N-CAM) (60). Sulfated PGs and GAGs interact with growth factors and cytokines, and serve as anchors for these molecules on the cell surface. Heparin and other sulfated GAGs (HS, CS, DS) can potentiate the ability of NGF, aFGF, and bFGF to induce neurite outgrowth in vitro (61–63). In the extracellular matrix, PGs may act as an axonal barrier (64–68). For example, PGs, through their capacity to interact

with neurotrophic factors, may establish regions containing high concentrations of neurite-promoting factors, diffusion from which creates gradients that may be followed by growing axons. Interestingly, the recent study of Nurcombe et al. *(69)* showed a developmental regulation of neural response to FGFs by HSPG. These authors showed that a single type of HSPG undergoes a rapid and tightly controlled change in FGF-binding specificity concomitantly with different temporal expression of two species of FGF. This change in specificity may be the result of a different glycosylation of this HSPG *(69)*.

PGs may also have a function in synaptic differentiation. They are involved in the formation of acetylcholine receptor clusters *(70–72)*. PGs have also been postulated to anchor acetylcholinesterase to the synaptic basal lamina *(73,74)* and to anchor nerves to their targets in electric organs *(75,76)*. Finally, the PGs associated with cerebral blood vessels play a fundamental role in maintaining the integrity of the blood–brain barrier *(77)*.

Proteoglycans in Alzheimer's Disease

GAGs and/or PGs have been found to be closely associated with all types of amyloidoses studied thus far *(32–36,78)*. In AD, histochemical analyses have provided evidence for the presence of PGs/GAGs in neuritic plaques, cerebrovascular amyloid, and neurofibrillary tangles. Two types of PGs, HSPG and DSPG, have been identified in neuritic plaques *(30,79–81)* and neurofibrillary tangles *(31,79,82)*. Unlike HSPG, which tends to be evenly distributed throughout plaques, the DSPG (decorin) was primarily localized to the periphery of the amyloid plaques *(79)*. The deposition of HSPG in amyloid plaques is an early event in the formation of the plaques and occurs prior to the appearance of fibrillar amyloid *(82,83)*. The origin of HSPG within amyloid plaques is not fully understood. Snow et al. *(82)* suggested that HSPGs are synthesized and deposited in the developing plaques by neurons and astrocytes.

Although the presence of PGs and GAGs in amyloid deposits is well established, their role in the pathogenesis of AD remains unclear. Most of the functions attributed to PGs and GAGs in the pathogenetic processing of AD relate to their contribution in amyloid fibril formation and deposition. By ELISA binding assay and by affinity chromatography, it has been shown that HSPG binds with high affinity to *βAPP (84)* and to the A4 peptide *(85,86)*. The interaction of HSPG with *βAPP* or A4 involved both the core protein and the GAG side chains *(84,86)*. Using the consensus sequence described by Cardin and Weintraub *(87)*, potential GAG binding sites have been identified on *βAPP (88)*. Of interest is the fact that one of these heparin-binding consensus sequences is localized on the A4 peptide within the nonamyloidogenic cleavage site. Recently, Baskin et al. *(89)* demonstrated that PGs can affect the secretory pathway of *βAPP*. These authors observed an increase of the nonamyloidogenic cleavage of *βAPP* after incubation of NGF-stimulated PC12 cells with PNP (*p*-nitrophenylxyloside). PNP, an initiator of GAG chain formation, inhibits the linkage of the GAG chains to the core protein and increases the amount of free GAG chains in the extracellular milieu. The increase of the nonamyloidogenic cleavage of *βAPP* in the presence of PNP may consequently be the result of fewer PGs in the cell membrane or more free GAGs in the extracellular milieu. From these observations, it appears that PGs can influence the processing of *βAPP* probably through its binding to A4. Thus, pathological interactions of PGs with A4 may favor the processing of *βAPP* to amyloidogenic fragments.

Sulfated PGs can influence protein folding *(90,91)*. It has been demonstrated in inflammation-associated amyloidosis (AA amyloid) that heparan sulfate can influence the secondary structure of the amyloid precursor protein (SAA_2) to adopt a β-pleated sheet conformation, which is the characteristic protein conformation of amyloid deposits. This structural transformation was not observed with a closely related protein (SAA_1), which is not an amyloid precursor *(92)*. Further studies are needed to determine if GAGs and/or PGs can influence the conformation of the APP in AD. This is particularly important, since protein folding can influence the biological activity of proteins and/or their sensitivity to proteolytic degradation. For example, the association of SAA_2 or bFGF with GAGs protects these components from proteolytic digestion *(61,93)*. It has been suggested that the binding of HSPG to bFGF induces a conformational change in bFGF that is an essential prerequisite for its recognition by its receptor *(94)*.

Although the A4 peptide is able to self-aggregate, sulfated GAGs may enhance the polymerization of the A4 peptide into insoluble fibers. It has been

shown by electron microscopic studies that sulfate ions and HS promote extensive aggregation of the amyloid peptide. The sulfate binding along the fibrils would provide electrostatic bridges to additional fibrils and result in the formation of macrofibers *(95)*. PGs and GAGs may also contribute to the stability of the amyloid and its inaccessibility to proteolytic degradation.

In addition to their possible role in amyloid formation and deposition, PGs with neurotrophic activity may play a role in neuritic sprouting in AD. Su et al. *(81)* have shown HSGAG-positive neurites within both primitive and classical senile plaques, whereas HSGAG-positive neurites were rarely detected in diffuse plaques. The presence of HS within diffuse plaques may attract neurites to the plaques and thereby permit further plaque development to occur. HS may also play a role in the development of neurofibrillary tangles, since HS is associated with intracellular and extracellular neurofibrillary tangles *(31,81,82)*.

Is the Metabolism of Proteoglycans Altered by the Acute-Phase Response in Alzheimer's Disease Brains?

An early study reported that AD tissue contained elevated levels of GAGs when compared to the brain of nondemented elderly individuals, suggesting that the GAG/PG biosynthesis is increased in AD *(96)*. Other investigators showed binding of bFGF to brain tissue sections from AD patients and not in controls, indicating the presence of HS in senile plaques and neurofibrillary tangles *(97)*. bFGF binds specifically to HS through the sequence GlcUA-GlcNSO$_3$-[IdoUA(2SO$_4$)-GlcNSO$_3$]$_5$-IdoUA-GlcNAc, and a negative correlation between 6-*O*-sulfate HS disaccharide content and bFGF binding to HS oligosaccharides has been reported *(98)*. In addition to the increased deposition of HS in senile plaques and neurofibrillary tangles, the excess binding of bFGF to AD brain may be the result of alterations in endogenous sulfation of HS. A change in the relative activity of enzymes responsible for the sulfation and epimerization of HS may favor the binding of bFGF to HS. For example, a decrease of the activity of the glucosaminyl-*N*-sulfate 6-*O* sulfotransferase and an increase of the activity of the iduronosyl 2-*O*-sulfotransferase may take place in tissues and cells isolated from AD patients *(99)*. The observation that the disulfated disaccharide ΔUA-GlcNSO$_3$-6SO$_4$ is decreased in fibroblasts isolated from AD patients is consistent with this hypothesis *(100)*. HS has been shown to be present in the cell nuclei of rat neurons *(101)* as well as in peripheral tissues, where it may be involved in regulation of gene expression *(60)*. Nuclear HS has a unique structure that is characterized by a higher ratio of disulfated disaccharides and a high proportion of glucuronic acid enriched in GlcUA-2SO$_4$ residues in the GAG chain *(102,103)*. An abnormally elevated level of HS in neuronal nuclei of AD tissues has been recently reported *(81)*. This increased amount of HS in neuronal nuclei may reflect an abnormally elevated degree of sulfation of neuronal HSGAGs.

From these observations, it appears that the metabolism of neuronal PGs is affected in AD. The alteration of PG metabolism may be the result of the cerebral acute-phase response described in AD *(16–19,104)*. Senile plaques are surrounded by reactive microglia and astrocytes *(17,105,106)*. IL-1, secreted by microglial cells and astrocytes, is involved in the brain inflammatory response *(107)*. For instance, an up to 30-fold increase in IL-1-immunoreactive glial cells, microglia, and astrocytes has been observed in tissue sections of AD brain *(108)*. This cytokine can induce the production of IL-6 and NGF *(109–111)*. The combined action of IL-1, IL-6, and NGF may be important in the increased amyloidogenesis in AD *(16,17,25)*. It has been shown that these three factors can promote the synthesis and the secretion of *βAPP (20,22–24)*. Moreover, NGF potentiates the neurotoxicity of the A4 peptide *(112)*.

We have studied the effect of IL-1 and NGF on the metabolism of secreted neuroblastoma PGs (snPGs). These snPGs, containing an HSPG, bind A4 peptide and share immunoreactivity with HSPG in amyloid plaques *(86)*. As we previously described *(86)*, neuroblastoma cells were metabolically labeled with [^{35}S]-sulfate and the labeled snPGs were purified by anion-exchange DEAE chromatography. IL-1 and NGF significantly increased the biosynthesis and the secretion of the labeled snPGs, as indicated by an increase in the amount of [^{35}S]-sulfate incorporation into the snPG fraction. In addition, the structure of the snPG glycosaminoglycans was altered by these treatments. An increase in the degree of sulfation of snPGs was observed as a result of NGF and IL-1 treatment (preliminary data, not shown).

Conclusion

In the brain, *βAPP*, the amyloid precursor protein of AD and Down's syndrome, is normally expressed at the cell surface or secreted into the extracellular media. It has been proposed that a pathologic amyloidogenic cleavage of *βAPP* resulted in the formation of A4 peptide and its subsequent accumulation in amyloid plaques *(4,5,22)*. However, the demonstration of the presence of amyloid peptide in the cerebrospinal fluid of nondemented people and in the culture medium of cells in culture suggests that the secretion of the A4 peptide is a normal processing event *(6–11)*. In this case, dysregulation of the metabolism of *βAPP* may be involved in the sequence of events that leads to the formation of amyloid plaques.

The mechanism of formation of amyloid plaques is still unknown. From diffuse plaques, the plaques are thought to progress chronologically through primitive plaques, neuritic plaques, and core plaques. In diffuse plaques, the substances deposited (principally *βAPP*/A4 and HSPG) are in a soluble state, whereas the transition of diffuse plaques to primitive plaques is characterized by the appearance of fibrillary structures. Reactive astrocytes are also observed within very primitive plaques *(17,105,106)*, and it has been proposed that the formation of very primitive/diffuse plaques starts around a central microglial cell *(113,114)*. The activation of microglial cells around the plaques will result in the liberation of cytokines and growth factors, including IL-1, IL-6, and NGF. In vitro experiments have demonstrated that these components stimulate the synthesis of *βAPP (20,22–24)*. An increase of *βAPP* synthesis has also been observed in AD brains *(13–15)*.

In AD, PGs have been found associated with amyloid plaques at their earliest stage of development, and with neurofibrillary tangles *(27)*. High-affinity interactions occur between HSPG and *βAPP*/A4 *(84–86)*. A heparin-binding consensus sequence has been demonstrated within the non-amyloidogenic cleavage site of the *βAPP* molecule *(88)*. In nonpathological conditions, the binding between *βAPP*/A4 and HSPG may physiologically regulate the functions of these molecules, for example, in neural cell adhesion and synapse formation *(22,115,116)*. However, the physiologic roles of HSPGs in regulating *βAPP* function are currently poorly understood. Cytokines and growth factors may simultaneously alter the metabolism of not only *βAPP*, but also neuronal HSPGs. We found that the metabolism of snPGs, which bind A4 and are present in senile plaques *(86)*, is altered by IL-1 and NGF. Such modifications of snPG metabolism, particularly in terms of GAG sulfation, may differentially alter snPG binding to *βAPP* and may favor amyloidogenic processing of *βAPP*. For example, as a result of NGF effects, enhanced binding of highly sulfated HSPGs with A4 may prevent normal proteolytic cleavage and favor the accumulation of the A4 peptide into insoluble aggregates. The A4 peptide aggregated into the amyloid deposits may, by its neurotoxic effect, stimulate glial activity. The attraction of microglia to the amyloid plaques may result in the local liberation of factors, such as IL-1 and NGF, which further stimulate the increased synthesis of *βAPP* and altered HSPGs, which will promote amyloidogenesis and plaque formation. A vicious cycle of amyloid formation can thus be proposed, initiated by local inflammation and attempts at neuronal repair.

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