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Multimodal signaling by the ADAMTSs (*a d*isintegrin *a*nd *m*etalloproteinase with *t*hrombo*s*pondin motifs) promotes neurite extension

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Abstract

Aggregating proteoglycans (PG) bearing chondroitin sulfate (CS) side chains associate with hyaluronan and various secreted proteins to form a complex of extracellular matrix (ECM) that inhibits neural plasticity in the central nervous system (CNS). Chondroitinase treatment depletes PGs of their CS side chains and enhances neurite extension. Increasing evidence from *in vivo* models indicates that proteolytic cleavage of the PG core protein by members of the ADAMTS (*a d*isintegrin *a*nd *m*etalloproteinase with *t*hrombo*s*pondin motifs) family of glutamyl-endopeptidases also promotes neural plasticity. The purpose of this study was to determine whether proteolytic action of the ADAMTSs influences neurite outgrowth in cultured neurons. Transfection of primary rat neurons with ADAMTS4 cDNA induced longer neurites, whether the neurons were grown on a monolayer of astrocytes that secrete inhibitory PGs or on laminin/poly-L-lysine substrate alone. Similar results were found when neurons were transfected with a construct encoding a proteolytically inactive, point mutant of ADAMTS4. Addition of recombinant ADAMTS4 or ADAMTS5 protein to immature neuronal cultures also enhanced neurite extension in a dose-dependent manner, an effect demonstrated to be dependent on the activation of MAP ERK1/2 kinase. These results suggest that ADAMTS4 enhances neurite outgrowth via a mechanism that does not require proteolysis but is dependent on activation of the MAP kinase cascade. Thus a model to illustrate multimodal ADAMTS activity would entail proteolysis of CS-bearing PGs to create a loosened matrix environment more favorable for neurite outgrowth, and enhanced neurite outgrowth directly stimulated by ADAMTS signaling at the cell surface.

Keywords: Plasticity; Protease; Primary neuron; Proteoglycan; Extracellular matrix; Matrix metalloproteinase; Cell culture

Introduction

Injury to the central nervous system (CNS) is often debilitating and compounded by little hope of recovery owing to the fact that once neural networks in the CNS are severed, they are difficult to re-establish. Predominantly, this is because the properties of myelin-associated proteins and other proteins that compose a glial scar impede the growth of axons towards their target. The glial scar is an accumulation of reactive astrocytes and extracellular matrix (ECM) molecules such as chondroitin sulfate (CS)-substituted PGs, tenascin and other proteins that inhibit the re-growth of axons and the migration of certain cells into the damaged region (Davies et al., 1999, 1997; Laywell et al., 1992). Indeed, the CS side chains of PG molecules are classical inhibitors of neurite outgrowth both *in vitro* and *in vivo* (Carulli et al., 2005, Silver and Miller, 2004; Snow et al., 2001).

Lecticans is the term for the family of hyaluronic acid-binding PGs that regulate cell adhesion, migration and neurite outgrowth in the CNS and include brevican, aggrecan, neurocan and versican (Handley et al., 2006). Long unbranched, sulfated, highly negatively-charged CS chains are covalently bound to the central domain of lecticans and discourage growth cone motility and neurite elongation. However, even when these glycosaminoglycan polymers are removed from the core protein

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by chondroitinase treatment (Pizzorusso et al., 2002), significant neurite inhibition is retained by versican (Schmalfeldt et al., 2000), but not by brevican (Miura et al., 2001). The enduring biological action may be inherent to the core PG protein itself or it may result from interactions with other ECM molecules such as hyaluronan or tenascin-*R*. *In vivo*, intermolecular interactions among lecticans, hyaluronan and tenascin result in the formation of a mesh-like lattice in the matrix of the CNS that inhibits neural plasticity (Fig. 1A). To facilitate plasticity, there should be a means to relieve the inhibition afforded by the PG. However, the absence of an endogenous, extracellular chondroitinase to remove CS chains is a limiting factor. So exploiting a mechanism that occurs *in vivo* may be a feasible way to re-establish plasticity in the brain. Increased expression and activation of endogenous proteases that cleave the PG core would be one mechanism to enhance neural plasticity by loosening the association and interaction among the matrix components that inhibit plasticity (Yamaguchi, 2000) (Fig. 1B).

The ADAMTSs (*a d*isintegrin *a*nd *m*etalloproteinase with *t*hrombo*s*pondin motifs) are multi-domain, metalloproteinases that have notable roles in angiogenesis, collagen processing, blood coagulation, cell migration, and arthritis, and several family members are glutamyl-endopeptidases that cleave lecticans (Porter et al., 2005). These secreted proteases share similar functional

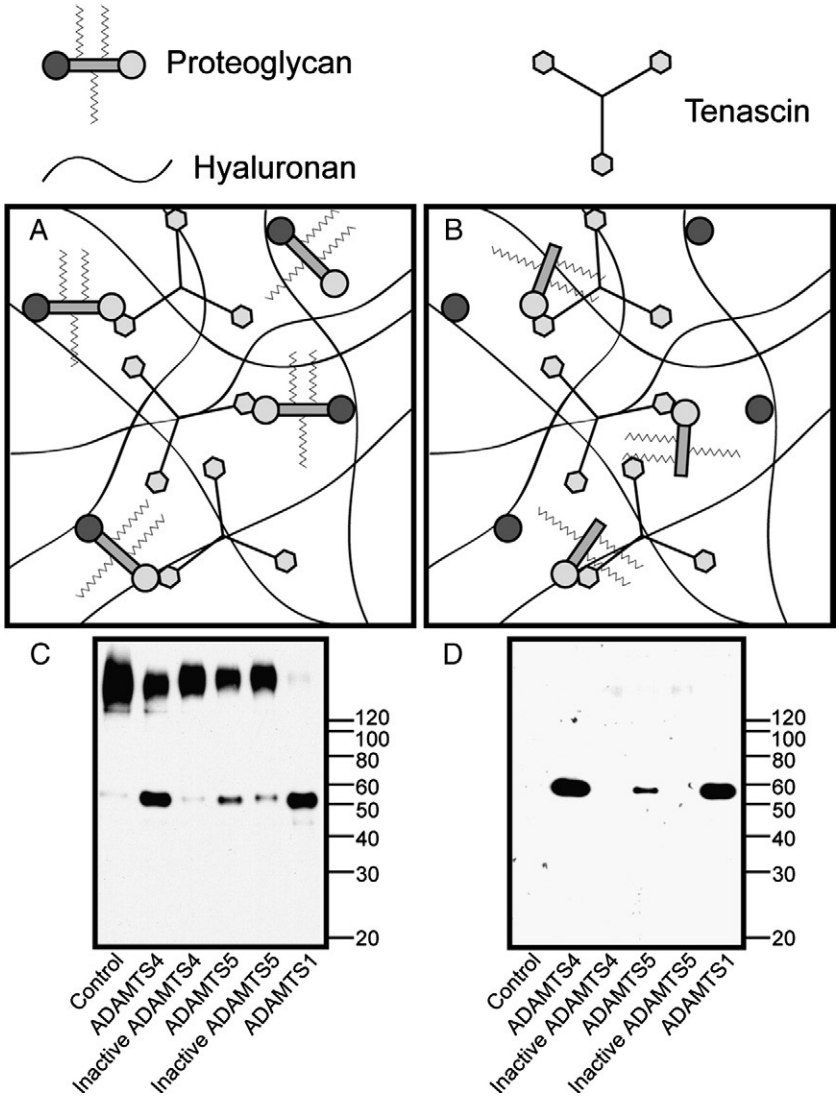

Fig. 1. Proteoglycan (lectican)–tenascin–hyaluronan matrix complex in the CNS and lectican cleavage by ADAMTSs. (A), Intact complexes of extracellular matrix form an inhibitory boundary toward neurite outgrowth by hyaluronic acid binding to the N-terminus, tandem repeats of the lecticans and tenascin binding to the C-terminus of the lecticans. (B), Cleavage of lectican by an ADAMTS loosens the stable, matrix network, potentially increasing fluidity and allowing for increased plasticity. (C, D), Cleavage of the lectican, brevican, by human recombinant ADAMTSs. Proteoglycans were purified from rat brain extracts contained in fractions eluted from a DEAE column with 1 M NaCl. Lectican-containing fractions were incubated with active ADAMTSs, or heat-inactivated protease (Inactive), subjected to SDS-PAGE, and probed with anti-brevican antibody (C) to reveal holoproteins and the N-terminal cleavage product or with anti-EAVESE antibody to selectively reveal the N-terminal fragment that contains the EAVEAE neoepitope exposed by ADAMTS-cleavage of brevican (D). "Control" lane shows brevican immunoreactivity as a smear at a molecular weight of >145 kDa. Samples containing inactive ADAMTS show little cleavage, whereas brevican substrate incubated with human recombinant ADAMTSs 1, 4 and 5 (25 nM) for 3 h reveal an abundance of the 55 kDa, N-terminal cleavage fragment (C) that is observed when probed with antibody selective for the ADAMTS-cleaved fragment (D).