Neurobiology of Aging xxx (2017) 1–13

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Antibody recognizing 4-sulfated chondroitin sulfate proteoglycans restores memory in tauopathy-induced neurodegeneration

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ARTICLE INFO

Article history:
Received 28 March 2017
Received in revised form 21 June 2017
Accepted 2 August 2017

Keywords:
Perineuronal nets
CSPGs
Object recognition memory
Plasticity
Alzheimer's disease

ABSTRACT

Chondroitin sulfate proteoglycans (CSPGs) are the main active component of perineuronal nets (PNNs). Digestion of the glycosaminoglycan chains of CSPGs with chondroitinase ABC or transgenic attenuation of PNNs leads to prolongation of object recognition memory and activation of various forms of plasticity in the adult central nervous system. The inhibitory properties of the CSPGs depend on the pattern of sulfation of their glycosaminoglycans, with chondroitin 4-sulfate (C4S) being the most inhibitory form. In this study, we tested a number of candidates for functional blocking of C4S, leading to selection of an antibody, Cat316, which specifically recognizes C4S and blocks its inhibitory effects on axon growth. It also partly blocks binding of semaphorin 3A to PNNs and attenuates PNN formation. We asked whether injection of Cat316 into the perirhinal cortex would have the same effects on memory as chondroitinase ABC treatment. We found that masking C4S with the Cat316 antibody extended long-term object recognition memory in normal wild-type mice to 24 hours, similarly to chondroitinase or transgenic PNN attenuation. We then tested Cat316 for restoration of memory in a neurodegeneration model. Mice expressing tau with the P301S mutation showed profound loss of object recognition memory at 4 months of age. Injection of Cat316 into the perirhinal cortex normalized object recognition at 3 hours in P301S mice. These data indicate that Cat316 binding to C4S in the extracellular matrix can restore plasticity and memory in the same way as chondroitinase ABC digestion. Our results suggest that antibodies to C4S could be a useful therapeutic to restore memory function in neurodegenerative disorders.

1. Introduction

Memory loss is a largely unmet medical challenge. Halting brain degeneration would be the most effective form of treatment, but it has yet to be achieved. An alternative is to enable the brain to function despite the degenerative damage. Plasticity enables the brain to bypass damage, but it is limited in adults by inhibitory proteoglycans in the brain extracellular matrix (ECM) situated in perineuronal nets (PNNs), which surround the key regulatory neurons.

PNNs are dense pericellular ECM structures with a structure similar to cartilage that develop at the time of closure of critical periods for plasticity. PNNs are a highly organized complex, mainly composed of hyaluronan, chondroitin sulfate proteoglycans (CSPGs), link proteins, and tenascin-R. The form of PNN most studied is those surrounding parvalbumin-positive (PV) interneurons recognized by the lectin Wisteria floribunda (WFA), although other forms of PNN exist around pyramidal and other neurons (Matthews et al., 2002). In the cortex, PNNs surround PV interneurons which are known to influence plasticity and excitability, and PNNs are involved in the termination of plasticity at the

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0197-4580/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2017.08.002

end of critical periods (Fawcett, 2015; Pizzorusso et al., 2002; Sugiyama et al., 2009). PNNs are also involved in memory. Depletion of chondroitin sulfate (CS) with the CS-digesting enzyme, chondroitinase ABC (ChABC) prolonged object recognition (OR) memory in normal mice (Romberg et al., 2013), restored lost memory in a neurodegeneration model in tauopathy mice and Abeta transgenic mice (Vegh et al., 2014; Yang et al., 2015), and affected fear memory extinction (Gogolla et al., 2009), demonstrating that CSPGs are the key molecules in regulating this form of memory and that the CS glycosaminoglycan (GAG) chains digested by ChABC are their effectors. Animals deficient in the PNN component Crtl1 link protein have very attenuated PNNs and show the same changes in memory and plasticity as animals treated with ChABC despite having unaltered CSPGs in the central nervous system (CNS), demonstrating that it is CSPGs in PNNs that are controllers of plasticity and memory (Carulli et al., 2010; Romberg et al., 2013). However, in order to reactivate plasticity, ChABC has to be injected directly into the brain parenchyma where it digests a region of up to 1 mm across, and the effect lasts for around 3 weeks. ChABC is therefore not a practicable treatment for memory restoration in neurodegenerative diseases. This study investigates antibody treatment as an alternative.

CSPGs and PNNs are implicated in neurodegenerative conditions in various ways. CSPGs are abundant in amyloid and neurofibrillary tangles in Alzheimer's disease (AD; reviewed in DeWitt et al., 1993; Kwok et al., 2011) and differentially sulfated CS such as chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S), and unsulfated chondroitin were detected (DeWitt et al., 1993). A reduction of PNNs in brains affected by AD was shown by staining with lectin WFA (Baig et al., 2005; Kobayashi et al., 1989), although many markers were not affected in AD patients and animal models of AD (Morawski et al., 2010b, 2012b). PV interneurons are largely unaffected by neurofibrillary tangles (Baig et al., 2005; Bruckner et al., 1999; Hartig et al., 2001; Morawski et al., 2010a, 2012b). This suggests that PNNs may be protective, and there is evidence for regulation of ion homeostasis (Suttkus et al., 2014) and neuroprotection against oxidative stress (Cabungcal et al., 2013; Suttkus et al., 2016). Treatment with chondroitinase does not affect hyperphosphorylated or filamentous tau (Yang et al., 2015), but an effect on Abeta quantity has been reported (Howell et al., 2015).

The functional effect and binding properties of CSPGs are heavily dependent on the patterns of sulfation of their GAG chains (Lin et al., 2011; Malavaki et al., 2008; Miyata and Kitagawa, 2015). The sugars in the GAG chain can be sulfated in the 4, 6, 2 + 6, and 4 + 6 positions on the CSs (C4S, C6S, C2,6S, and C4,6S, also known as CS-A, CS-C, CS-D, and CS-E), respectively, and different patterns of sulfation give motifs that can provide specific binding sites (Mikami and Kitagawa, 2013; Xu and Esko, 2014; Yabuno et al., 2015). C4S is inhibitory to neurite growth while C6S is more permissive to neurite growth and to plasticity (Lin et al., 2011; Miyata et al., 2012; Wang et al., 2008). Thus, mice with a knockout of chondroitin 6-sulfotransferase 1 showed worse regeneration and less plasticity than wild-type (WT) animals (Lin et al., 2011) while chondroitin 6-sulfotransferase 1 overexpressing mice demonstrated enhanced plasticity into adulthood (Miyata et al., 2012). During embryonic development C6S predominates, but at the end of the critical period in the PNN fraction only 4.9% of CS-GAG is 6-sulfated while 80.6 % is the inhibitory 4-sulfated form (Carulli et al., 2010; Deepa et al., 2006). We have recently demonstrated that this trend continues into aging when memory deficits start to appear. C6S was reduced almost to zero in aged rats (12 and 18 months old) when compared to young animals (Foscarin et al., 2017). Moreover, the sulfation pattern of PNN CS-GAGs (which make up 2% of total brain CS-GAG) is different from that of the general brain matrix, giving the PNN specific binding properties (Deepa et al., 2006). Thus, semaphorin 3A (Sema3A) and OTX2 bind specifically to PNNs but not to the general matrix (Beurdeley et al., 2012; Dick et al., 2013; Miyata et al., 2012; Vo et al., 2013), where they are positioned to affect synapse dynamics and PV cell maturation. Both these molecules bind most strongly to 4,6 disulfated CS-GAG. The major CSPG core protein of PNNs is aggrecan, which is necessary for PNN formation (Carulli et al., 2016; Dick et al., 2013; Giamanco et al., 2010; Morawski et al., 2012a) and contains specific glycan modifications in its linkage region (Yabuno et al., 2015). The CS-GAGs carried by aggrecan are probably the key component of PNNs that regulate memory and plasticity, because animals lacking aggrecan in the CNS show the same memory prolongation phenotype as animals with attenuated PNNs due to Crtl1 knockout or ChABC treatment (unpublished observations) (Romberg et al., 2013). Our hypothesis therefore was that masking C4S on aggrecan would make PNNs less inhibitory and would therefore have a similar effect as ChABC in restoration of memory to brains damaged by neurodegeneration or in prolonging memory in normal animals.

Seeking potential treatments for memory restoration, we have tested an inhibitory proteoglycan neutralizing antibody. In this manuscript, we have identified Cat316 as a C4S binding antibody that can modulate the inhibitory properties of the glycan, reduce Sema3A binding, prolong OR memory in normal animals, and restore lost memory in tauopathy animals.

2. Materials and methods

2.1. Screening of PNN-blocking antibodies using PNN-HEK cells

PNN-human embryonic kidney (HEK) cells (~ 12,000 cells) were plated on poly-L-lysine–coated coverslips for 2 hours and cultured in low-serum medium (0.5 % fetal bovine serum in Dulbecco's modified eagle medium [DMEM]) in the presence of 5% of CO_2. Detached cells were then removed with a rinse in fresh warm medium. Potential PNN-blocking antibodies (Cat316 is from Millipore; HAPLN1 is from R&D; 6B4 and 7B7 are from CosmoBio) were diluted into 5 μg/mL in cultured medium before incubation with the PNN-HEK cells for 48 hours at 37 °C with 5% of CO_2. Control treatment with ChABC (200 mU/mL for 30 minutes) was done after plating. Then, cells were rinsed in warm culture medium and stained with anti-aggrecan antibody (1:400, rabbit polyclonal, Millipore) for 30 minutes for PNNs. Cells were then fixed with 4% paraformaldehyde before incubation with secondary anti-rabbit antibodies. Cells were counterstained with Hoechst and coverslipped with anti-fading medium FluoroSave (Calbiochem) before visualizing using Leica confocal microscope.

For experiments testing the efficiency of Cat316 in blocking Sema3A from binding to the PN-HEK cells, PNN-HEK cells were cultured as mentioned previously, and Sema3A (40 ng/mL) was added to the culture medium for 2 hours at 37 °C. The cells were then rinsed with warm DMEM twice for 10 minutes each at 37 °C before being processed for immunocytochemistry of Sema3A using a protocol as mentioned previously.

2.2. Quantification of optical density of aggrecan staining in PNN-HEK cells

All images were captured with the same settings using a Leica confocal microscope (with 1 frame at numerical aperture 1.15, 405 nm laser [12%], 488 nm laser [38%], and 532 nm laser [38%]). The red channel corresponding to aggrecan staining of the PNNs was converted into black and white images. Optical density of the images was measured using ImageJ software (1.49v, National Institutes of Health, USA). The data were then normalized first to a negative control (omitting primary antibodies), before further normalization against the positive control (i.e., phosphate buffered