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Diverse functions of perineuronal nets

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Perineuronal nets represent well-organised components of the extracellular matrix, which are surrounding cell bodies, dendrites, and axon segments of a particular class of neurones as well as forming lattice-like structures. The role of perineuronal nets is not fully elucidated yet. Perineuronal nets may play a beneficial role by stabilizing the extracellular milieu assuring the characteristic features of enveloped neurons and protecting them from the influence of harmful agents. On the other hand, perineuronal nets create a barrier which limits neuronal plasticity and counteracts regeneration. This review examines recent evidence concerning the significance of the occurrence of perineuronal nets.

Key words: proteoglycans, extracellular matrix, chondroitinase ABC, neuronal plasticity, neuronal regeneration, GABAergic interneurons, parvalbumin

INTRODUCTION

The extracellular matrix (ECM) of the central nervous system (CNS) contains an extremely complex mixture of proteoglycans, tenascin, fibronectin, and a scaffolding substance - hyaluronan. These components can be diffusely dispersed in the neuropil or they can surround cell bodies, dendrites, and axon segments of neurons, forming well-organised lattice-like structures (Deepa et al. 2006). These structures were first described by Camillo Golgi (1873) and later they were named perineuronal nets (PNs). PNs can be observed in virtually all regions of the CNS but only subsets of neurons are enveloped by these specific forms of ECM.

The main component of PNs, proteoglycans, can be categorised according to the nature of their glycosaminoglycan (GAG) chains attached to the core protein. These chains are formed with heparin sulphate (HS), keratin sulphate (KS), and chondroitin sulphate (CS). CS proteoglycans, known as lecticans, are the most abundant in the CNS. They are represented by nervous system specific proteoglycans: neurocan, brevican, and phosphacan (Margolis et al. 1996, Yamaguchi 1996). There are also proteoglycans such as aggrecan and versican that were found in the nervous system and in the connective tissues (for review see Yamaguchi 2000). It should be stressed that PNs display extremely high heterogeneity because of variety of GAG chains attached to the core proteins. Additionally, there are different isoforms of the core proteins. Since sulphate and uronic groups are often attached to the GAG chains, this makes the microenvironment surrounding the neurons well-hydrated and strongly anionic. However, there is evidence that intensively positive charged nets can also be found (for review see Murakami and Ohtsuka 2003). Hypothetical models of PNs structure have been proposed in several reviews (Dityatev and Schachner 2003, Murakami and Ohtsuka 2003, Rauch 2004, Galtrey and Fawcett 2007).

METHODS OF PNS VISUALIZATION

Camillo Golgi, at the end of the nineteenth century, used the silver chromate precipitates method, which allowed him to observe PNs. Various staining methods have recently been applied to visualize PN-like structures. Among them binding with agglutinins from *Wisteria floribunda* (WFA), *Vicia villosa* (VVA) and soy bean (SBA) are the most frequently used methods to detect PNs (Seeger et al. 1996, Tsubouchi et al. 1996, Guerrero-Tarrago et al. 1999). These plant lectins bind selectively and with high affinity to the terminal alpha and/or beta

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N-acetylogalactosamine residues. Proteoglycans are sulphate glycoconjugates, therefore they can be detected by using anionic or cationic iron colloid; this allows differentiation between various groups of neurons with negatively or positively charged PNs (Murakami et al. 2001). The PNs can also be stained with Kopsh's silver nitrate or methylene blue that visualizes the core proteins of proteoglycans (Murakami et al. 1999). Nowadays, immunohistochemical staining methods are widely used to localize components of PNs (Fig. 1A, B).

Antibodies used for detection of PNs components reveal difficulties in studying these complex lattice-like structures. For example, at late stages of postnatal development of mice, CAT-315 antibody detects glycan present on aggrecan, whereas at the early postnatal period, CAT-315-reactive CS proteoglycan is a glycoform of receptor protein tyrosine phospatase beta (RPTPβ) (Dino et al. 2006), which is related to phosphacan (Meyer-Puttlitz et al. 1995). Although antibody CAT-301 and CAT-315 detect the same core protein of CS proteoglycan, they stain different subpopulations of neurons, i.e., pyramidal and nonpyramidal neurons, respectively (Ojima et al. 1998), because they recognize distinctly glycosylated epitopes on aggrecan (Lander et al. 1997, Mathews et al. 2002).

REGIONAL AND CELLULAR DISTRIBUTION OF PNS

Staining studies show the differences in the distribution and composition of PNs throughout the CNS. This concerns both species and regional variations (Ojima et al. 1998, Brückner et al. 1998, 2003, Ishii and Maeda 2008, Nakamura et al. 2009). The PNs are unevenly distributed in brain regions. In the mature CNS of humans, rats and mice, PNs are related to the motor and motor-related structures (Bertolotto et al. 1996, Mabuchi et al. 2001). Abundance of PNs is observed in the visual and somatosensory cortex, especially in layer IV of the barrel cortex of mice, rats, and Mongolian gerbils, where sensory signals from whiskers are received (Brückner et al. 1994, Nowicka et al. 2003, Bahia et al. 2008, Nakamura et al. 2009).

The structure differences of proteins and carbohydrate components of CS proteoglycans suggest that they may envelop different types of neurons (Deepa et al. 2006). In deep cerebral nuclei, the brain stem, as well as in the spinal cord, PNs surround projection neurons (Bertolotto et al. 1996, Carulli et al. 2006). In the rat neocortex, staining with WFA reveals three types of neurons (Wegner et al. 2003). Most of the PNs surround a non-pyramidal subset of neurons which express markers specific for inhibitory GABAergic (γ-aminobutyric acid-ergic) interneurons. Among them parvalbumin (PV) positive cells represent the most frequent group and they are widely distributed across all cortical layers (Fig. 1C). The other two types of neurons described by Wegner and coauthors (2003) reveal faintly labelled PNs. One of them represents glutamate-positive excitatory pyramidal cells with PNs weakly stained by WFA, whereas the second type has diffuse extracellular matrix nets and are immunoreactive for both glutamatergic and GABAergic markers. They are classified as specific modified pyramidal cells.

Some degree of specificity exists in the composition of PNs surrounding different types of neurons. Indeed, although unsulphated chondroitin proteoglycan is involved in the meshwork structure around GABAergic interneurons, some of components of PNs are not present (Bertolotto et al. 1996). The versican 1 isoform, which belongs to the lectican family, does not colocalize with WFA stained cells and its immunoreactivity is absent on GABAergic interneurons. Glycosaminoglycan-α (GAGα) immunoreactivity, characteristic for versican, is located mainly on large projection neurons rather than interneurons (Horii-Hayashi et al. 2008).

Experiments performed on frozen sections from mouse brains and *in vitro* studies on organotypic mouse midbrain slice culture show that PNs may envelope not only the somatic and proximal parts of dendrites but also axonal initial segments, extending as far as the beginning of the myelin sheath in large motoneurons of the mice (Brückner et al. 2006) and cat spinal cord (Hockfield et al. 1990) as well as in the mouse superior colliculus (Brückner et al. 2006).

The components of PNs are generated by two types of cells: neurons and glia. Glia produces tenascin, versican, and hyaluronan, whereas neurons produce other CS proteoglycans such as phosphacan and brevican (Seidenbecher et al. 2002, Hayashi et al. 2005). Neurocan is principally produced by reactive astrocytes, however, neurons are also able to synthesize this proteoglycan (Engel et al. 1996, Matsui et al. 2002, Jones et al. 2003). Experiments on primary cerebral neuronal cultures obtained from rats demonstrated that aggrecan is also produced by neurons (Lander et al. 1998, Matthews et al. 2002). It should be stressed,

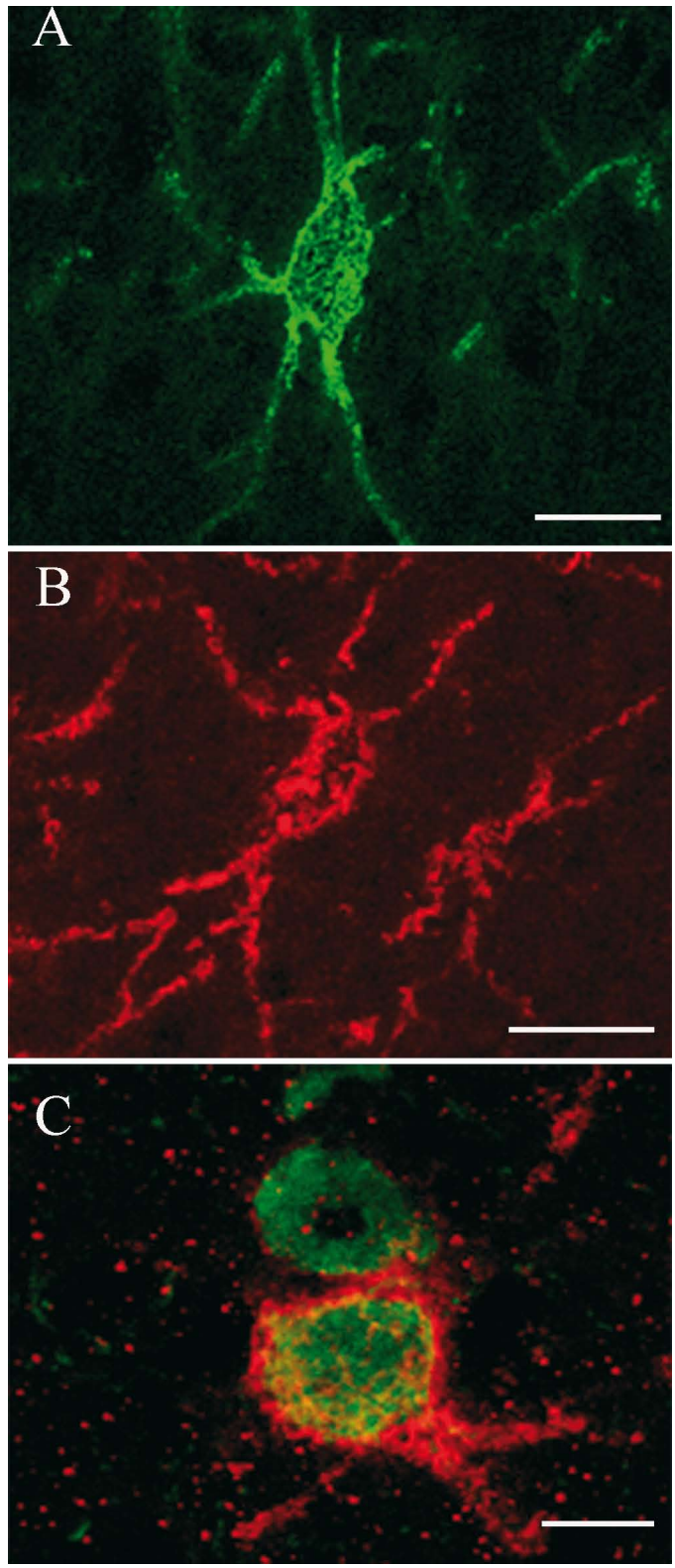

Fig. 1. Representative neurons enveloped by PNs from layer IV of the mouse somatosensory cortex. This figure shows: (A) Cell stained for PNs with WFA (green), Scale bar is 25 μm; (B) Cell stained for aggrecan with CAT-315 antibody (red), Scale bar is 25 μm; (C) Double staining for PV (green) and PNs visualized with WFA (red). Note that only one of two neighbouring PV-positive cells is enveloped by PNs. Scale bar is 10 μm.

however, that PN-like structures are able to appear without the presence of glial cells (Miyata et al. 2005) and dissociated hippocampal neurons in culture can form net-like ECM (John et al. 2006, Dityatev et al. 2007, Frischknecht et al. 2009).

Formation of PNs depends on neuronal activity. Blocking of sodium channels by tetrodotoxine inhibits the formation of PNs in experiments performed on slices from the mouse visual cortex (Reimers et al. 2007). On the other hand, these studies show that suppression of glutamatergic transmission by antagonists of metabotropic and ionotropic glutamatergic receptors does not significantly alter the density of neurons enveloped by PNs. Thus, neuronal activity is required for the development of PNs, however, their formation is independent of neuronal glutamatergic system (Reimers et al. 2007).

DEVELOPMENT OF PNs

The complicated and highly charged PNs structure creates a defined and stable microenvironment around neurons in the adult CNS. However, the composition of extracellular matrix changes during development. In mice, the juvenile form of matrix is replaced by the mature form at about 2–5 weeks after birth and this meshwork is maintained throughout adulthood (for review see Zimmermann and Dours-Zimmermann 2008). At early postnatal stage, mammalian PNs contain mostly neurocan and versican 1 (Milev et al. 1998). In the adult CNS, other members of lectican family such as aggrecan, versican 2, and brevican prevail (for review see Zimmermann and Dours-Zimmermann 2008). In the rat cerebellum, proteoglycans visualized by WFA staining appear at the same time as other components of PNs: aggrecan, hyaluronan, and cartilage link protein (Crtl1). Up-regulation of mRNAs of these three components, when the nets start to form, indicates that they may be the most important molecules for the condensation of PNs around neurons (Carulli et al. 2007).

A question arises concerning which of numerous components of PNs are indispensable for PNs formation. Studies on tenascin-R and double tenascin-R/parvalbumin knockout mice (KO-mice) reveal morphological alterations in the PNs network around interneurons (Weber et al. 1999, Haunso et al. 2000). PNs found in these KO-mice around neuron soma show disrupted, punctuated staining and the labeling around dendrites is absent. A loss of CS proteoglycans such as phosphacan and neurocan is also reported. These results indicate that tenascin-R is essential for ECM proteoglycans to form the lattice-like structure (Weber et al. 1999, Haunso et al. 2000).

It appears that precise timing of the CS proteoglycans expression seems to be specific for both cortical areas and particular cortical layers. For example, the developmental time course of WFA-detected proteoglycans and aggrecan immunoreactivity visualized by CAT-315 antibody differs in the somatosensory and visual cortices. In the visual cortex, the density of cells with aggrecan- positive PNs remains almost constant from P10 to the adulthood. This observation contrasts with neurons enveloped by PNs, revealed by WFA lectin binding, which show gradual increase of WFA-positive neurons during development. In the somatosensory cortex, however, density of cells stained with WFA and aggrecan-positive cells gradually increase from P10 until adulthood. Interestingly, in the visual cortex, aggrecan can be detected already at P10, whereas proteoglycans detected with WFA cannot be found yet. These data suggest that aggrecan can be an important element for the initial accumulation of PNs around neurons (Karetko et al. 2008).

ROLE OF IMMATURE PNs

One of possible roles of the immature PNs is the formation of the link between the extracellular space and intracellular cytoskeleton (for review see Celio and Blumcke 1994, Wintergerst et al. 1996). Parvalbumin-containing GABAergic interneurons display special composition of cytoskeleton proteins, i.e., $ankyrin_R$ and $\beta_R$spectrin. These intracellular membrane-related cytoskeleton proteins appear during postnatal development before the PNs components are detected. This observation may suggest that the degree of cytoskeleton maturity may influence PNs formation (Wintergerst et al. 1996).

It has been also proposed that at early stages of development, specific properties of immature PNs components attract and trap neurotrophic substances, e. g., growth factors, and by this means they may regulate maturation of neurons and stimulate axonal growth. For example, basic fibroblast growth factor (bFGF), responsible for neuronal survival and neurite extension, is found in PNs (for review see Celio and Blumcke 1994). It can be speculated that proteoglycans present

in PNs can act as low affinity receptors that concentrate neurotrophic factors in the vicinity of neurons. Because some growth factors can interact *in vivo* with components of ECM, these interactions could be essential for PNs activity (Flaumenhaft and Rifkin 1991).

Some PNs components are expressed before a peak of postnatal synaptogenesis and can be important for synapse formation (Milev et al. 1998, Popp et al. 2003). Results of *in vitro* studies support this idea since aggrecan-immunoreactive puncta appear on the surface of neurons in primary neuronal cultures before synapse formation. This aggrecan appearance and accumulation at the presumed extrasynaptic sites suggests that their distribution can delineate the location of future synapses (Dino et al. 2006).

ROLE OF MATURE PNs

As postnatal maturation of CNS proceeds, PNs become more complex and start to tightly envelop neurons (for review see Rauch 2004). The mature extracellular meshwork has repellent properties against approaching new nerve fibres and growth cones (McKeon et al. 1991, Niederost et al. 1999, for review see Rhodes and Fawcett 2004), and it is well documented that proteoglycans and tenascin-R inhibit adhesion between neurons (Grumet et al.

Table I

Effect of Chondroitinase ABC treatment on neuronal plasticity

Species	Place of ChABC action	Effect of PNs degradation	References
mouse	hippocampal slices	reduction of LTP	Bukalo et al. 2001
rat	axotomized nigrostriatal pathway	long-distance regeneration of interrupted dopaminergic nigral axons	Moon et al. 2001
rat	visual cortex	restoration of ocular dominance plasticity in adult	Pizzorusso et al. 2002, 2006
rat	injured spinal cord	functional recovery of locomotor and proprioceptive behaviors	Bradbury et al. 2002
rat	injured spinal cord	sprouting of injured and intact spinal cord projections	Barrit et al. 2006
rat	injured spinal cord	regeneration of damaged dorsal root fibers	Cafferty et al. 2007
rat	injured spinal cord	sprouting of intact dorsal root fibers; restoration of sensory function	Cafferty et al. 2008
mouse	amygdala	abolishment of spontaneous recovery and context-dependent renewal of conditioned fear in adult	Gogolla et al. 2009
mouse	amygdala	impairment of LTP at thalamo-LA inputs	Gogolla et al. 2009
rat	culture of hippocampal neurons	increase of lateral diffusion of AMPA receptors	Frischknecht et al. 2009

(ChABC) chondroitinase ABC; (LA) lateral amygdala; (LTP) long term potentiation; (PNs) perineuronal nets

1985, Pesheva et al. 1993). Thus, PNs create a barrier against the formation of the new synaptic contacts.

Maturation of PNs can effectively reduce a plastic potential of neurons, which suggest a role of PNs in stabilization of synaptic contacts. Mature forms of PNs occur rather late during postnatal life and their appearance coincides with the end of experience-dependent plasticity, i.e., the onset of some critical periods (Pizzorusso et al. 2002, 2006, McGee et al. 2005, for review see Hensch 2005). To elevate plastic properties within the CNS, chondroitinase ABC (ChABC), which degrades PNs and therefore reduces inhibitory CS proteoglycans features, was used in different experimental models (Crespo et al. 2007, Kwok et al. 2008). Studies by Pizzorusso and colleagues (2002) demonstrate that treating the mature rat visual cortex with ChABC restores ocular dominance plasticity, and this implies that the maturation of PNs inhibits neuronal plasticity in the visual cortex. In line with this concept are the results of Gogolla and others (2009). They show that a coincidence exists between developmental "switch" in the PNs maturation in the basolateral amygdala and in the susceptibility to erasure of fear memories of adult mice. Moreover, the degradation of PNs by ChABC in amygdala abolishes spontaneous recovery and context-dependent renewal of conditioned fear. These data lead the authors to an intriguing concept that PNs protect fear memories from erasure (Gogolla et al. 2009). Additional information concerning the effect of PNs removal on synaptic plasticity is provided by experiments with long-term potentation (LTP). In slices obtained from ChABC-injected mice, LTP of monosynaptic excitatory inputs in the thalamo-lateral amygdala is completely abolished.

In vitro degradation of PNs by ChABC interferes with induction of LTP and long-term depression (LTD) in hippocampal slices (Bukalo et al. 2001). The elegant studies of Frischknecht and coworkers (2009) performed on rat hippocampal neurons in culture using hyaluronidase or ChABC to remove PNs reveal that a net-like structure of ECM influence mobility of α-amino-3-hydroxy-5-methyl-4 isoxazoleproprionic acid (AMPA) receptors and creates a barrier for a local diffusion of AMPA receptors on dendrites. Thus, PNs form compartments on the neuronal surface that control the accessibility of local receptor populations to the synapse (Frischknecht et al. 2009). Morover, after PNs removal by hyaluronidase, synaptic short plasticity is also altered as revealed in pair-pulse experiments. Taken together, these data strongly support a view that mature PNs counteract synaptic plasticity.

The presence of PNs may also interfere with reinervation processes occurring in the CNS after injury. Application of ChABC after unilateral nigrostriatal axotomy in rat brains promote long-distance regeneration of interrupted dopaminergic nigral axons (Moon et al. 2001). In the spinal cord, CS proteoglycans are present in both white and grey matter (Tang et al. 2003). These PNs molecules are widely distributed in the dorsal column pathway of the spinal cord (for review see Kaas et al. 2008). As mentioned above, CS proteoglycans are inhibitors of axonal growth and their expression is elevated after spinal cord injury, limiting somatosensory plasticity in the dorsal column pathway (Silver and Miller 2004, Massey et al. 2007). Injection of ChABC to the spinal cord in the vicinity of a lesion site degrades CS proteoglycans and promotes sprouting of injured dorsal column axons and causes functional recovery (Bradbury et al. 2002). Moreover, after spinal cord injury, degradation of CS proteoglycans can promote sprouting not only of the injured spinal projections but also of the intact ones. This observation suggests an additional mechanism of functional recovery after ChABC post-injury treatment (Barritt et al. 2006). Recent studies have gone further and showed that in behavioral recovery after spinal cord injury a reorganisation of the connectivity of the intact root is involved rather than the regeneration of damaged dorsal root fibres. The presence of one intact root is sufficient to restore sensory function (Cafferty et al. 2007, 2008). Table I summarizes results of the ChABC experiments.

The restoration of plasticity in the adult brain is possible not only after degradation of the PNs components using ChABC or hyaluronidase but also when PV-positive cells are devoid of the influence exerted by Otx2 homeodomein protein. This protein binds to a promotor region of tenascin-R, and thus this can directly regulate PNs formation and maturation (Sugiyama et al. 2008).

In the barrel cortex, a part of the primary somatosensory cortex of rodents, appearance of PNs at the third postnatal week around interneurons in the interbarrel space can create compartments and stabilizes connections formed earlier, and therefore can limit plasticity. When unilateral sensory deprivation of whiskers for three weeks is performed in rats at early postnatal age, *Viccia villosa* agglutinin (VVA) labeling

pattern was diffuse in a hemisphere with restricted sensory inputs. Cells with VVA-positive PNs were rarely seen in layer IV, moreover the barrel field pattern was also disturbed. Thus, there is an interaction between extrinsic, sensory activity, and architectural aspects of PNs formation during development (Bahia et al. 2008). In the rodents' barrel cortex, normal development of the PNs pattern depends on peripheral stimulation of whiskers. Sensory deprivation of newborn mice alters sensory inputs but only reduces aggrecan expression in the barrel field (McRae et al. 2007). Latest studies indicate, however, that deprivation of one row of whiskers in mice causes a decrease in number of PNs-enveloped cells in the barrel hollows but the number of PNs-enveloped neurons in the barrel septa remain unchanged. This indicates that thalamic input promotes maturation of GABAergic neurons within the barrel hollows (Nakamura et al. 2009). In the barrel cortex, plasticity can also be evoked by single whisker rearing without a time limit except for layer IV, where it can only be induced during the first postnatal week. After such procedure, in the deprived barrels neighbouring the barrel representing intact whiskers, an increased number of PV-positive cells and increased numbers of PNs-enveloped cells are found. These data suggest the involvement of PNs in closing the critical period in layer IV in an experience-dependent manner (Nowicka et al. 2009). In line with the concept that the appearance of mature PNs closes the critical periods of pasticity are the most recent results showing that the sensitive period for vocal plasticity in zebra finch is regulated by PNs (Balmer et al. 2009).

A notion that PNs may play a role in synapse stabilisation is strengthened by observations that close connections exist between neurons and astrocytic protrusions which may additionally stabilize synaptic contacts. A direct topographic relation of astrocytic processes to net-like structures on neuronal somata and dendrites is reported by Derouiche and coauthors (1996). Different proportions of PNs components around these two cellular components can create specific neuronal environment which can form a perisynaptic "barrier" and permanently stabilize synaptic contacts (for review see Celio and Blumcke 1994, Murakami and Ohtsuka 2003).

Another important role of PNs is related to their anionic feature and their role in maintenance of local ion homeostasis in a neuronal environment. GABAergic interneurons enveloped by PNs are less susceptible to a harmful accumulation of lipofuscin as compared to delicately enveloped pyramidal cells. These observations suggest a protective function of CS proteoglycans against oxidative stress induced by metal ions: Al, Fe, Cu, and Zn. Components of PNs can interact predominantly with Fe, which is involved in generation of highly reactive hydrogen radicals (Reinert et al. 2003, Morawski et al. 2004, Canas et al. 2007). PNs have also a protective role against excitotoxic environment. After trimethyltin intoxication, CS proteoglycan-immunoreactive PNs and PNs-enveloped interneurons persist in the severely affected hippocampus and the dentate gyrus of rats. These experiments also show that activated microglia do not degrade PNs associated with interneurons (Schuppel et al. 2002). CS proteoglycans can reduce delayed neuronal death induced by excitotoxic concentrations of glutamate (Okamoto et al. 1994). Taken together, these results indicate that CS proteoglycans may have a neuroprotective action in acute harmful conditions in the brain.

Proteoglycan-containing PNs are resistant to proteolytic degradation by trypsin, chymotrypsin, or pepsin. However, if brain tissue sections were earlier treated with ChABC and hyaluronidase to disrupt carbohydrate residues, these proteolytic enzymes are able to digest CS proteoglycans present in PNs. These findings suggest a possible role of carbohydrate residues of PNs as cofactors enhancing the activity of protease inhibitors (Carrell et al. 1987, Koppe et al. 1997). Therefore PNs are supposed to protect cells against harmful proteolytic action enhanced after insult. This protective role of PNs is demonstrated in the experiments involving peripheral nerve injury in which tenascin-R and PNs was shown to prevent neurons from activated microglia (Angelov et al. 1998).

It is reported that PNs generate and maintain a polyanionic, ion-buffered microenvironment. As mentioned above, PNs surround predominantly PV-containing GABAergic interneurons. These cells are classified as fast-firing neurons which display specific electrophysiological properties. High activity of these neurons is possible due to cation channels, especially a subunit of voltage-gated potassium-channels, Kv3.1b. Since the localization of these channels overlaps not only with interneurons in numerous rat brain regions but also with a distribution pattern of PNs, it has been hypothesised that the strongly anionic microenvironment can be involved in cation turnover (Brückner et al. 1993). Large degree of co-distribution

of Kv3.1b channels, parvalbumin and PNs staining also occurs in the monkey (*Macaca mulatta*) cortex (Hartig et al. 1999). Taken together, it appears that PNs can prevent free diffusion of potassium and sodium ions.

PNs are also involved in maintenance of water homeostasis since they contain hydrophilic molecules (mostly CS proteoglycans and hyaluronan). Layer I of the neocortex as well as the molecular layer of the hippocampus shows an inverse correlation of aquaporin 4 with the distribution of aggrecan or hyaluronan suggesting a complementary role of PNs and aquaporin 4 in the maintenance of water homeostasis. Highly hydrophilic components of PNs allow easier circulation of water throughout the extracellular space, whereas in areas with tighter cellular contacts aquaporin 4 can facilitate water diffusion (Costa et al. 2007).

Experiments with knock-out (KO) mice provide additional evidence for a role of PNs. Mutant mice lacking functional proteoglycans, such as neurocan, brevican, aggrecan, and tenascin-R reveal several abnormalities during development, in behavior and CNS function. Mice with inactive brevican gene display lower expression of other PNs components, particularly neurocan, and express problems with a maintainance of a late-phase of the hippocampal LTP (Brakebush et al. 2002). However, neurocan-deficient mice show no behavioral or functional deficits (Zhou et al. 2001). Other studies performed on brevican- and neurocan-deficient mice after rhizotomy indicate that these proteoglycans contribute to a non-permissive CNS environment and make regeneration of nerve fibres difficult (Quaglia et al. 2008). Tenscin-R deficits trigger robust brain dysfunction, anatomical and behavioral changes. *In vivo* studies on tenascin-R-deficient mice show behavioral impairment such as a decrease in cognitive and motor skills (Freitag et al. 2003, Montag-Sallaz and Montag 2003). In adult tenascin-C-deficient mice plasticity of cortical whisker representation after vibrissectomy is reduced (Cybulska-Klosowicz et al. 2004).

Electrophysiological studies show that absence of tenascin-R results in reduction of LTP and short-term depression after stimulation of Schaffer's collateral in the CA1 hippocampal field (Bukalo et al. 2001). Tenascin-R is an important constituent of PNs-enveloped PV-positive interneurons which mediate perisomatic inhibition. The absence of tenascin-R reduces perisomatic inhibition in the CA1 field in tenascin-R-deficient mice *in vitro* (Nikonenko et al. 2003), *in vivo* (Gurevicius et al. 2005) and causes increased basal excitatory synaptic transmission. It has been proposed by Sykova and others (2005) that tenascin-R could play a crucial role in maintaining optimal extracellular space between synapses and thus can regulate volume transmission.

PNs AND NEUROPATHOLOGY

There is some evidence of loss and alterations in PNs composition in various degenerative diseases; however, it is not clear whether some relationship exists between PNs and CNS disorders. PNs could be involved in human prion diseases such as Creutzfeldt-Jacob disease (CJD) or scrapie disease that affects animals (Caughey and Raymond 1993, Vidal et al. 2006). These encephalopathies are characterised by imbalance and vulnerability of GABAergic inhibitory interneurons, which could be the result of previous destruction of PNs. At early stages of CJD, a reduction of extracellular meshwork takes place before disappearance of PV-positive neurons. In contrast, in scrapie-affected sheep brains, PNs are preserved but simultaneously a reduction of PV-positive neurons is observed (Vidal et al. 2006). In scrapie affected cells, inhibitory interaction exists between CS proteoglycans and protein resistant prion protein (Prp-res) which preserves cells against the accumulation of Prp-res in amyloidal plaques (Caughey and Raymond 1993).

Differences in the PNs composition appear in another neurodegenerative disorder, Alzheimer's disease (AD). Although there is no co-appearance of WFA-positive PNs with markers of AD such as tau protein, amyloid β-peptide, and MHC class II antigen (marker of activated microglia), a substantial loss of negatively charged N-acetylgalactosamine (GalNac) residues of PNs-enveloped cells is shown. PNs maintain ion homeostasis around neurons therefore their loss is likely to impair primarily function of GABAergic neurons (Baig et al. 2005). Since PNs can exert a protective role, their dysfunctions may facilitate accumulation of amyloid plaques. The treatment of cultured cortical neurons with amyloid β peptide shows that PNs-enveloped neurons survive whereas PNs-free neurons die (Miyata et al. 2007). In transgenic mouse (Tg2576) model of AD, aggrecan-expressing neurons are not affected by formation of amyloid plaques (Morawski et al. 2008). Moreover neurons enveloped by PNs are less susceptible to cytoskeleton changes in AD. Double stained sections from human brains show that pyramidal and non-pyramidal neurons enveloped

by PNs are unaffected by the formation of neurofibrillary tangles even in severely damaged regions (Brückner et al. 1999). Abnormal phosphorylation of tau protein, which is one of the AD markers, occurs in cells devoid of PNs in the aged cerebral cortex (Hartig et al. 2001). It should be stress that other studies suggest a neuropathological role for PNs in AD. It is well established that HS proteoglycans lead to hyperphosphorylation of tau protein and to neurofibrillar deposit formation, a unique feature observed in AD (Goedert et al. 1996). Experiments showing that PNs take part in transformation of amyloid beta into the pathological, secondary structure argue against the protective role of PNs in AD (Hilbich et al. 1991).

Profound alterations in proteoglycans of ECM are found in patients with multiple sclerosis. Enhanced lectican expression in the proinflamatory milieu of expanding lesion edges and lectican decreases in plaque centre may influence the failure to support neurite outgrowth axonal regeneration and remyelination (Sobel and Ahmed 2001). Latest studies indicate that in cerebral cortical regions affected by multiple sclerosis the loss of WFA-positive PNs was due to the elevation of metalloproteinase 9 (MMP-9, enzyme that degrade extracellular matrix) and to impairments of PV-positive cells resulting from the accumulation of phosphorylated neurofilament protein (Gray et al. 2008).

Cerebral ischemia enhances neurogenesis in the adult brain (for review see Kokaia and Lindvall 2003). This phenomenon is accompanied by activation of MMPs (Wojcik et al. 2009). This suggests that the degradation of PNs by MMPs may participate in the regulation of ischemia-induced neurogenesis. It is well established that recovery after stroke is limited. Investigations performed on animal models show that in the infarct core borders activated astrocytes form the glial scar. This process is accompanied by elevation of the growth-inhibiting CS proteglycans (Carmichael et al. 2005, Hobohm et al. 2005, Beck et al. 2008, Shen et al. 2008). However, there is a loss of PNs expression in peri-infarct and remote areas after both a photothrombotic stroke (Bidmon et al. 1998) and a middle cerebral artery occlusion (MCAO) (Hobohm et al. 2005) performed in rats. In these regions remodeling of neuronal networks take place therefore down regulation of CS proteoglycans can promote repair processes (Carmichael et al. 2001, 2005, Hobohm et al. 2005). In case of MCAO this loss is permanent (Hobohm et al. 2005). After photothrombotic stroke however, after longer survival period (30 days) PNs start to reappear. In areas remote from infarct core, PNs are restored faster, and as early as one week after photothrombosis reaches the control levels (Karetko et al. 2009).

CONCLUSION

Perineuronal nets appear to play an important role in synaptic stabilisation and plasticity, ion homeostasis and neuroprotection. Present data show that a disruption of PNs by chondroitinase ABC might restore the plastic potential of neurons both in the intact and injured CNS by promoting nerve fibre growth and reinstalling connectivity. These create possibilities to develop new therapeutic strategies to improve functional recovery after brain and spinal cord injury. The only other problem that emerges is the involvement of PNs in neurodegenerative diseases.

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