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BRAIN RESEARCH

Research Report

Chondroitin sulfate proteoglycan-based extracellular matrix in chicken (*Gallus domesticus*) brain

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ABSTRACT

A specialised form of extracellular matrix consisting of large aggregating chondroitin sulphate proteoglycans connected to hyaluronan and tenascins, as main components, is termed perineuronal nets. These perineuronal nets surround subpopulations of neurons in many vertebrates including man. In this study we investigated the distribution and the postnatal development of perineuronal nets in the brain of the domestic chicken using immunohistochemical, lectin-histochemical and biochemical methods. Perineuronal nets could be identified very early, already on the first postnatal day throughout various regions and nuclei in chicken fore- and midbrains, most expressively in nidopallium, hyperpallium, lateral striatum, globus pallidus and mesopallium. These mostly delicate, scanty structures around the cell bodies of neurons thicken and complete during the first 2 weeks, however, differ in shape and clearness of contours from the mature form of perineuronal nets found in the adult, 3 year old animals. Perineuronal nets frequently co-localized with the potassium channel subunit Kv3.1b characteristic for fast spiking neurons but remained unrevealed around cholinergic or monoaminergic neurons. The early appearance of perineuronal nets in the precocial birds' brain is probably due to the rapid establishment of neuronal morphology and function which is required for the immediate functional and behavioural performance of chicken.

1. Introduction

Previous studies have investigated the accumulations of extracellular matrix components around neuronal perikarya in mammalian brains. These structures, called perineuronal nets (PNs), ensheath the perikaryon, proximal parts of dendrites as well as the axon initial segment of the nerve cells (Brückner et al., 2006a) and are associated with different types of neurons in region-specific patterns in many vertebrates including man (Brückner et al., 1993; Oohira et al., 1994; Seeger et al., 1994; Wegner et al., 2003; for review see Celio et al., 1998). The biological significance of PNs is not clear and several functions have been proposed. Through the inhibitory potential to cell adhesion and the repulsive properties of some

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Table 1 – Primary antibodies, lectins and protein used in this study.

	Company	Species	Dilution	Reference
Antibodies				
Cat-315	Millipore	Mouse, mc[a]	1:1000	Matthews et al. (2002)
1-B-5	ICN	Mouse, mc[a]	1:800	Brückner et al. (2008)
Anti-human CRTL-1 (hHAPLN1)	R&D Systems Inc.	Goat, pc[b]	1:400	Carulli et al. (2007)
Anti-aggrecan AB1031	Millipore	Mouse, mc[a]	1:1000	Brückner et al. (2006)
Anti-aggrecan HAG7D4	Acris	Mouse, mc[a]	1:10	Brückner et al. (2008)
Anti-choline acetyltransferase (ChAT)	Millipore	Goat, pc[b]	1:100	Brückner et al. (2008)
Anti-tyrosine hydroxylase (TH)	Millipore	Rabbit, pc[b]	1:1000	Brückner et al. (2008)
Anti Kv-3.1b	Alomone Labs.	Rabbit, pc[b]	1:2000	Härtig et al. (1999)
Lectins				
Wisteria floribunda agglutinin (WFA)	Sigma	Biotinylated	1:50	Härtig et al. (1992)
Helix aspersa agglutinin (HAA)	Sigma	Biotinylated	1:50	
Vicia villosa agglutinin (VVA)	Sigma	Biotinylated	1:50	Härtig et al. (1992)
Protein				
Hyaluronan-binding-protein (B-HABP)	Cape Cod	Biotinylated	1:50	Delpech et al. (1989)

[a] Monoclonal.
[b] Polyclonal.

of their molecular components against approaching axons and dendrites PNs might contribute to the stabilization of ensheathed synaptic contacts, thereby reducing their neuroplastic potential (Pizzorusso et al., 2002; Berardi et al., 2003; Dityatev and Schachner, 2003; Rhodes and Fawcett, 2004). Other functions of PNs might be related to their polyanionic character. The glycosaminoglycan chains of PNs provide highly charged structures in the direct microenvironment of neurons that might be involved in local ion homeostasis. They can, thus, potentially act as buffering system for physiologically relevant ions such as calcium, potassium and sodium around highly active types of neurons (Brückner et al., 1993, 1996a, 1996b; Härtig et al., 1999; Reinert et al., 2003; Morawski et al., 2004). Recent investigations have suggested that the specialised microenvironment created by PNs have neuroprotective effects (Morawski et al., 2004; Wu et al., 2005; Morawski et al., 2008). PNs are molecularly heterogeneous, consisting primarily of chondroitin sulphate proteoglycans (CSPG) of the lectican family (aggrecan, versican, neurocan and brevican) complexed with hyaluronan and tenascin-R (Köppe et al., 1997; Brückner et al., 1998, 2000; Yamaguchi, 2000; Matthews et al., 2002; Rauch, 2007). In this regard aggrecan is the predominant neuronal CSPG in adult mammalian brain (Matthews et al., 2002). This finding is in agreement with findings about the neuronal allocation of aggrecan in embryonic chick brain (Schwartz et al., 1996; Domowicz et al., 2003). Several of these CSPGs are first expressed at the end of the period of synapse formation, leading to the suggestion that the elaboration of PNs might be an important element in developmental modification of synapses which might potentially contribute to the stabilization of the ensheathed synaptic contacts (Hockfield et al., 1990; Pizzorusso et al., 2002).

In precocial birds, neuronal differentiation and the development of synaptic contacts start very early and proceed rapidly during postnatal life which is due to the required, practically immediate functional performance of the animal (Tömböl, 1988, 1995). The dendritic arbour of neurons shows considerable maturity right after hatching, already on the first postnatal day (P1) with high, although not maximal spine density typical for adult animals (Tömböl, 1988, 1995). Actually, neuronal morphology and function appear to be equally mature throughout the whole rostrocaudal extension of the brain, i.e. neurons in rostral telencephalic regions are similarly well differentiated to those in the brain stem surprisingly early in postnatal life. This is in accordance with recent advances that showed high expression of tenascin-C (TN-C) transcripts in the chick forebrain in the first postnatal week which includes the critical period for auditory filial imprinting (Metzger et al., 2006). TN-C immunoreactivity was particularly accumulated around parvalbumin positive neurons in a pattern that is reminiscent of perineuronal nets of the extracellular matrix (Metzger et al., 2006).

Up to now no description or mapping has been made about the distribution of PNs in the avian brain. The aim of the present study was (i) to give an overall description of PNs in the chicken forebrain, midbrain and cerebellum during postnatal development and (ii) to investigate whether the time course of the appearance of PNs corresponds to the early maturation of neuronal morphology. Perineuronal nets in different mammalian species have been reliably labelled with antibodies against CSPGs (Matthews et al., 2002; Bertolotto et al., 1991; Lander et al., 1998; Wegner et al., 2003; Adams et al., 2001; Brückner et al., 2006b), N-acetylgalactosamine-binding lectins such as *Wisteria floribunda* agglutinin (WFA) (Härtig et al., 1992, 1999; Brückner et al., 2006b) or were detected histochemically using the colloidal iron hydroxide-(CIH)/Prussian blue reaction (Murakami et al., 1993; Seeger et al., 1994; Reinert et al., 2003, Morawski et al., 2005). Investigations of the present study were based on these in mammals' well established markers (Table 1).

2. Results

Chondroitin sulphate proteoglycan-based extracellular matrix detected with Cat-315 and 1-B-5 immunohistochemistry