Please cite this article in press as: Kecskes S et al. Extracellular matrix molecules exhibit unique expression pattern in the climbing fiber-generating precerebellar nucleus, the inferior olive. Neuroscience (2014), http://dx.doi.org/10.1016/j.neuroscience.2014.09.080

Neuroscience xxx (2014) xxx–xxx

EXTRACELLULAR MATRIX MOLECULES EXHIBIT UNIQUE EXPRESSION PATTERN IN THE CLIMBING FIBER-GENERATING PRECEREBELLAR NUCLEUS, THE INFERIOR OLIVE

S. KECSKES,[a†] B. GAÁL,[a,b†] É. RÁCZ,[a] A. BIRINYI,[a] A. HUNYADI[c] AND C. MATESZ[a,b,c*]

[a] *Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

[b] *Division of Oral Anatomy, Faculty of Dentistry, University of Debrecen, Debrecen, Hungary*

[c] *MTA-DE Neuroscience Research Group, Debrecen, Hungary*

Abstract—Extracellular matrix (ECM) accumulates around different neuronal compartments of the central nervous system (CNS) or appears in diffuse reticular form throughout the neuropil. In the adult CNS, the perineuronal net (PNN) surrounds the perikarya and dendrites of various neuron types, whereas the axonal coats are aggregations of ECM around the individual synapses, and the nodal ECM is localized at the nodes of Ranvier. Previous studies in our laboratory demonstrated that the heterogeneous distribution and molecular composition of ECM is associated with the variable cytoarchitecture and hodological organization of the vestibular nuclei and may also be related to their specific functions in gaze and posture control as well as in the compensatory mechanisms following vestibular lesion. Here, we investigated the ECM expression pattern in the climbing fiber-generating inferior olive (IO), which is functionally related to the vestibular nuclei. By using histochemical and immunohistochemical methods, the most characteristic finding was the lack of PNNs, presumably due to the absence of synapses on the perikarya and proximal dendrites of IO neurons. On the other hand, the darkly stained dots or ring-like structures in the neuropil might represent the periaxonal coats around the axon terminals of olivary synaptic glomeruli. We have observed positive ECM reaction for the hyaluronan, tenascin-R, hyaluronan and proteoglycan link protein 1 (HAPLN1) and various chondroitin sulfate proteoglycans. The staining intensity and distribution of ECM molecules revealed a number of differences between the functionally different subnuclei of IO. We hypothesized that the different molecular composition and intensity differences of ECM reaction is associated with different control mechanisms of gaze and posture control executed by the visuomotor-vestibular, somatosensory and integrative subnuclei of the IO. © 2014 Published by Elsevier Ltd. on behalf of IBRO.

Key words: hyaluronan, chondroitin sulfate proteoglycans, tenascin-R, link protein, vestibular system, brainstem.

INTRODUCTION

In the central nervous system (CNS), the extracellular matrix (ECM) molecules fill the extracellular space. The ECM is involved in neural plasticity, engaged in signal transduction pathways and influence the neuronal activity during normal and pathological conditions (Hartig et al., 1999; Bradbury et al., 2002; Dityatev and Schachner, 2003; Busch and Silver, 2007; Dityatev and Fellin, 2008; Kwok et al., 2008; Sykova and Nicholson, 2008; Zimmermann and Dours-Zimmermann, 2008; Morita et al., 2010; Kwok et al., 2011). The major components of the ECM are hyaluronan (HA), chondroitin sulfate proteoglycans (CSPGs) including aggrecan, brevican, neurocan, versican, and glycoproteins (GP) e.g., tenascin-R (TN-R) and link proteins (Margolis et al., 1975; Delpech et al., 1989; Eggli et al., 1992; Gong et al., 1994; Yasuhara et al., 1994; Dityatev and Schachner, 2003; Carulli et al., 2006). The ECM molecules may aggregate around various parts of the neurons and form the perineuronal net (PNN), the nodal ECM and axonal coats (ACs), or they are distributed throughout the neuropil (Koppe et al., 1997; Celio et al., 1998; Carulli et al., 2006; Deepa et al., 2006; Bruckner et al., 2008; Zimmermann and Dours-Zimmermann, 2008; Bekku et al., 2009; Bekku and Oohashi, 2010). As shown in various parts of the CNS, the molecular and structural heterogeneity of the ECM exhibits an area-dependent distribution pattern, which correlates with the cytoarchitecture, neurochemical properties, connection and function of the neurons (Matesz et al., 2005; Szigeti et al., 2006; Bruckner et al., 2008; Meszar et al., 2008;

*Correspondence to: C. Matesz, Department of Anatomy, Histology and Embryology, University of Debrecen, Nagyerdei krt. 98, Debrecen H-4032, Hungary. Tel: +36-52-255-567; fax: +36-52-255-115.
E-mail address: matesz@anat.med.unideb.hu (C. Matesz).

† The first two authors contributed equally to this work.

Abbreviations: β, nucleus beta; ACs, axonal coats; bHABP, biotinylated Hyaluronan Binding Protein; BSA, bovine serum albumin; bWFA, biotinylated *Wisteria floribunda* agglutinin; CNS, central nervous system; CSPGs, chondroitin sulfate proteoglycans; DAO, dorsal accessory olive; DC, dorsal cap; DMCC, dorsomedial cell column of inferior olive; ECM, extracellular matrix; GP, glycoprotein; HA, hyaluronan; HAPLN1, hyaluronan and proteoglycan link protein 1; HMAO, horizontal lamella of medial accessory olive; IgG, immunoglobulin G; IO, inferior olive; MAO, medial accessory olive; NGS, normal goat serum; NHS, normal horse serum; NRS, normal rabbit serum; PBS, phosphate-buffered saline; PNN, perineuronal net; PO, principal olive; RMAO, rostral lamella of medial accessory olive; RT, room temperature; TN-R, tenascin-R; VLO, ventrolateral outgrowth; WFA, *Wisteria floribunda* agglutinin.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.080
0306-4522/

Morawski et al., 2009; Gati et al., 2010; Lendvai et al., 2012; Morawski et al., 2012; Jager et al., 2013; Racz et al., 2013; Gaal et al., 2014).

In our previous study we have mapped the distribution, organization and molecular composition of ECM in the vestibular nuclei of brainstem, and observed a number of differences between the individual nuclei associated with their different functions (Racz et al., 2013). The vestibular nuclei receive labyrinthine, visual, and proprioceptive inputs and after complex information processing, the output is transmitted directly or indirectly to the eye moving and spinal motoneurons to maintain the posture and ocular stability during the head and body displacement also (Brodal, 1984; McCrea et al., 1987a,b). In the indirect transmission, the precerebellar nuclei have a pivotal role via the activation of the cerebellum through the mossy and climbing fiber system (Kitai et al., 1977; McCrea et al., 1977). In contrast to the various sources of the mossy fibers, the climbing fibers originate exclusively from the inferior olive (IO). The mammalian IO is composed of the principal olive (PO), dorsal (DAO) and medial accessory olive (MAO) subnuclei. In addition, minor subgroups of neurons such as the dorsal cap (DC), nucleus beta (β), ventrolateral outgrowth (VLO), and dorsomedial cell column (DMCC) are also distinguished (Kooy, 1917; Gwyn et al., 1977; Azizi and Woodward, 1987). Functionally, the olivary nuclei are divided into somatosensory, visuomotor-vestibular and integrative groups (Gwyn et al., 1977; De Zeeuw et al., 1998; Urbano et al., 2006; Azizi, 2007) and project to the cerebellum in topographically organized pattern (Brown, 1980; Azizi and Woodward, 1987; Barmack et al., 1998; De Zeeuw et al., 1994; Zguczynski et al., 2008; D'Angelo et al., 2011). Because the olivary subnuclei are functionally heterogeneous and have different afferent and efferent connections we suppose that it is reflected in different organization and composition of ECM. On the other hand, the morphologically and functionally uniform population of olivary neurons and the characteristic glomeruli throughout the entire neuropil (Gwyn et al., 1977; De Zeeuw et al., 1998) would suggest a homogeneous ECM pattern. At present, it is not known whether this contradiction is associated with a unique organization and composition of the ECM.

We have described for the first time that the peripheral vestibular lesion modifies the HA and CSPG expression pattern in the lateral vestibular nucleus of the rat, suggesting the involvement of ECM in the development of postlesional symptoms and in the vestibular compensation (Deak et al., 2012). The vestibular lesion also results in temporary and permanent morphological, molecular, and physiological changes in functionally related areas, for instance in the precerebellar nuclei (Balaban and Romero, 1998; Fukushima et al., 2001; Li et al., 2001; Tighilet et al., 2014). Knowledge on the organization and distribution of ECM molecules in the intact IO, as the goal of this paper, is required to follow the possible changes in the ECM expression to understand the role of ECM in the background of vestibular compensation, at system level.

EXPERIMENTAL PROCEDURES

Animals and tissue processing

The study protocol was reviewed and approved by the Animal Care Committee of the University of Debrecen, Debrecen, Hungary according to national laws and European Union regulations [European Communities Council Directive of 24 November 1986 (86/609/EEC)], and was properly conducted under the control of the University's Guidelines for Animal Experimentation (license number: 11/2011/DEMAB).

The experiments were performed on adult female (12–14-week-old) Wistar rats (n = 6) from Charles River Laboratory (Strain Crl:WI), weighing from 250 to 300 g each. The animals were terminally anesthetized with an intraperitoneal injection of 10% urethane (1.3 ml/100 g body weight; Reanal, Budapest, Hungary) and immediately perfused transcardially with physiological saline. The brainstems were removed and immersed into Sainte-Marie's fixative (99% absolute ethanol and 1% glacial acetic acid) for 1 day at 4 °C. The specimens were embedded in paraffin and transverse sections were made with a microtome at a thickness of 8 μm and two consecutive sections from the same animal were captured on one slide. Following deparaffination, sections were rehydrated and washed in phosphate-buffered saline (PBS), pH 7.4, and treated with 3% H_2O_2 for 10 min at room temperature (RT).

Histochemistry and immunohistochemistry

Prior to histochemical and immunohistochemical reactions, all specimens were blocked for 30 min at RT in 1% bovine serum albumin (BSA) (HA; *Wisteria floribunda* agglutinin, WFA), 1% BSA + 10% normal goat serum (NGS) (aggrecan), 2% BSA (versican; Hyaluronan and Proteoglycan Link Protein 1, HAPLN1), 3% normal horse serum (NHS) (neurocan), 1% BSA + 10% NHS (brevican), and 1% BSA + 10% normal rabbit serum (NRS) (TN-R).

Histochemical reactions. Distribution of HA was detected by applying biotinylated Hyaluronan Binding Protein (bHABP; 1:50; kindly provided by R. Tammi and M. Tammi, Dept. Anat. Univ. of Kuopio, Kuopio, Finland). WFA histochemistry was performed using biotinylated *Wisteria floribunda* agglutinin (bWFA; 1:500; Sigma–Aldrich, St. Louis, MO, USA), a lectin binding to N-acetylgalactosamine residues of CSPG-glycosaminoglycan chains and GP, as a marker of PNNs (Hartig et al., 1992). After blocking, sections were incubated in a solution of bHABP or bWFA, dissolved in PBS containing 1% BSA, overnight at 4 °C. Visualization of labeling was accomplished by incubating the samples with ExtrAvidin Peroxidase complex (Sigma–Aldrich) for 1 h at RT diluted in PBS followed by 3,3′-diaminobenzidine-tetrahydrochloride (DAB, Sigma–Aldrich) with H_2O_2. After dehydration, sections were coverslipped with DPX mounting medium (Sigma–Aldrich).

Please cite this article in press as: Kecskes S et al. Extracellular matrix molecules exhibit unique expression pattern in the climbing fiber-generating precerebellar nucleus, the inferior olive. Neuroscience (2014), http://dx.doi.org/10.1016/j.neuroscience.2014.09.080