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Extracellular matrix components mark the territories of circumventricular organs

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HIGHLIGHTS

- Extracellular matrix components are abundant in circumventricular organs.
- Their immunoreactivities generally mark the territories of circumventricular organs.
- Matrix is suggested to control access of blood-borne molecules to adjacent areas.

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ABSTRACT

In the central nervous system the extracellular matrix has important roles, e.g. supporting the extracellular space, controlling the tissue hydration, binding soluble factors and influencing their diffusion. The distribution of the extracellular matrix components in the brain has been mapped but data on the circumventricular organs (CVOs) is not available yet. The CVOs lack the blood–brain barrier and have relatively large perivascular spaces. The present study investigates tenascin-R and the lecticans: aggrecan, brevican, neurocan, and versican in the median eminence, the area postrema, the vascular organ of the lamina terminalis, the subfornical organ, the pineal body and the subcommissural organ of the rat applying immunohistochemical methods, and lectin histochemistry, using *Wisteria floribunda* agglutinin (WFA). The extracellular matrix components were found intensely expressed in the CVOs with two exceptions: aggrecan immunoreactivity visualized only neurons in the arcuate nucleus, and the subcommissural organ was not labeled with either WFA, or lecticans, or tenascin-R. The different labelings usually overlapped each other. The distribution of the extracellular matrix components marked the territories of the CVOs. Considering these we suppose that the extracellular matrix is essential in the maintenance of CVO functions providing the large extracellular space which is required for diffusion and other processes important in their chemosensitive and neurosecretory activities. The decrease of extracellular matrix beyond the border of the organs may contribute to the control of the diffusion of molecules from the CVOs into the surrounding brain substance.

1. Introduction

It has been proved that the central nervous system (CNS) has an extracellular matrix built up mainly from similar components as in other parts of the body although some components occur only in the CNS. The CNS extracellular matrix has an important role in brain development, cell migration, axon growth, synaptogenesis, brain vascularization, cell adhesion, tissue hydration, the binding of the soluble factors, and the control of their diffusion [1,2].

In the CNS the main extracellular matrix components are the hyaluronic acid, some glycoproteins (laminin, fibronectin, tenascin) and some proteoglycans consisting of a central core protein with glycosaminoglycan side chains, mainly chondroitin sulphates. The most abundant brain proteoglycans are the hyaluronan binding 'hyalectans', also called 'lecticans', as the versican, aggrecan, neurocan and brevican [3–6].

The CNS extracellular matrix is found dispersed in the neuropil [4], or forming perineuronal 'nets' [4,6–8].

The distribution of the extracellular matrix components in the brain has been mapped (for example see [4,9–11]) but data on the circumventricular organs (CVOs) are not available yet, except for the occurrence of laminin and fibronectin in the CVOs in general [12] and that of some components in the neurohypophysis [13,14].

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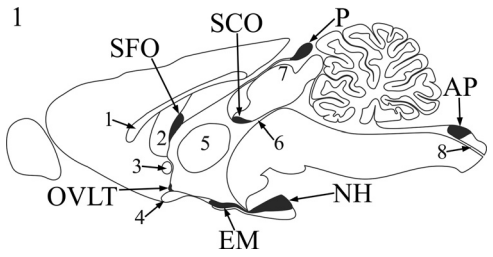

Fig. 1. Scheme on the positions of the CVOs mentioned in the text. Mediansagittal plane, based on the photography of [16]. AP – area postrema, EM – median eminence, OVLT – vascular organ of the lamina terminalis, NH – neurohypophysis, P – pineal body, SCO – subcommissural organ, SFO – subfornical organ.
Other structures to help orientation: 1 – corpus callosum, 2 – fornix, 3 – anterior commissure, 4 – optic chiasm, 5 – interthalamic adhesion, 6 – cerebral aqueduct, 7 – superior colliculus, 8 – central canal.

The CVOs are supposed to have special neurosecretory and/or chemosensitive functions, e.g. in the salt- and water-balance, the circulation, and the gastrointestinal functions. Therefore they require a free access to cerebral blood (a lack of the blood–brain barrier), and relatively large perivascular spaces. The subcommissural organ forms an exception having a complete blood–brain barrier. For recent reviews about the CVOs see [15–18] and for a scheme on their positions see Fig. 1.

The present study investigates the aggrecan, brevican, neurocan, versican and tenascin-R (the main tenascin of the mature brain) in the median eminence, the area postrema, the vascular organ of the lamina terminalis (OVLT), the subfornical organ, the pineal body and the subcommissural organ of the rat by immunohistochemical method. Lectin histochemistry with *Wisteria floribunda* agglutinin (WFA) which binds N-acetyl-D-galactosamine-bearing glycoconjugates of e.g. chondroitin sulphate-containing proteoglycans [9,19] was applied as well.

2. Materials and methods

Adult albino rats (Wistar) of either sex, of 250–300 g body weight, were used. All experimental procedures were performed in accordance with European Communities Council Directive (86/609/EEC) guidelines.

The animals were deeply anesthetized with ketamine and xylazine injections (20 and 80 mg/kg, respectively, intramuscularly) and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were postfixed in the same fixative for 1 day at 4 °C. Serial sections (50 μm thickness) in the coronal plane were cut by a vibration microtome.

Floating sections were pretreated with 20% normal horse serum for 90 min. Sections were incubated with primary immunoreagents (mouse, monoclonal anti-neurocan and anti-versican, both diluted 1:100, DSHB, Iowa City, IA, investigators: R. K. Margolis and R. A. Asher; goat, polyclonal anti-tenascin-R, 1:100, R&D Systems, Minneapolis, MN; mouse, monoclonal, anti-brevican, 1:100, BD Biosciences, Sparks, MD; rabbit, polyclonal, anti-aggrecan, 1:500, Merck Millipore, Billerica, MA in PBS containing 0.5% Triton X-100, and 0.01% sodium azide) for 40 h, then processed according to the avidin-biotinylated peroxidase method. For a detailed description see e.g. Kálmán et al. [20]. In the case of the WFA-binding (1:100, biotinylated, Vector Laboratories, Burlingame, CA) the 40 h incubation was followed immediately with the avidin-biotinylated peroxidase complex. Control sections were incubated by the omission of the primary immunoreagents. No structure-bound labeling was observed in these specimens.

Photomicrographs were taken by a DP50 digital camera mounted on an Olympus BX-51 microscope (both from Olympus Optical Co. Ltd., Tokyo, Japan).

3. Results

In the median eminence the territories marked by both tenascin-R and versican were very similar (Fig. 2a and b). The imunoreactivity extended from the ependyma to the pial surface where it interdigitated with deep unstained invaginations. Lateralward the immunolabeling had a border corresponding to the lateral corners of the bottom of the ventricle but along the ventricle the reactivity extended dorsally. In the posterior part of the organ this dorsal extension surrounded a narrow periventricular 'demilune' (Fig. 2b). The versican immunoreactivity was quite amorphous, whereas that of tenascin-R had a fibrous pattern perpendicular to the pial surface (see insets).

On the other hand, the distribution of brevican and that of neurocan were also similar (Fig. 2c and d). They decorated a middle zone, leaving the periventricular and subpial zones unstained. The staining intensity gradually decreased medialward. Laterally the immunoreactive zone diverged dorsal- and ventralward and closed laterally the thin-walled bottom of the third ventricle.

The WFA was bound throughout the median eminence (Fig. 2e). The territory of the intense binding extended over the ventromedial part of the arcuate nucleus at the border of the median eminence, unlike what was seen in the case of versican and tenascin-R. Here WFA binding delineated neurons, otherwise it was amorphous and continuous. In several other brain areas mainly in the cortex WFA binding also visualized neurons. Immunoreactivity of aggrecan also labeled neurons in the arcuate nucleus (Fig. 2f) but it was not found in CVOs.

In the pineal body two different patterns were found. In the case of WFA, a network was formed as if it delineated the cell borders (Fig. 2g). The vessels were delineated by the immunoreactivity of both tenascin-R and versican and neurocan and brevican, but only in the rostral part of the organ. Between the vessels thin immunopositive filaments were stretched. Of the similar immunopatterns only that of tenascin-R is shown in figure here (Fig. 2h).

In the subfornical organ the immunoreactivity of versican (Fig. 3a) was intense arranged in a mesh-like pattern throughout the organ leaving only a small innermost area unstained. Since the tenascin-R immunostaining resulted in a similar pattern, it is not shown in figure. The neurocan immunoreactivity (Fig. 3b) extended over the whole organ but it proved to be more intense along the border of the hippocampal commissure and around the large lateral vessels on both sides, as if it formed a 'shell' of the organ. A similar distribution was found in the case of brevican, therefore it is not shown in figure. The WFA-binding (Fig. 3c) was confined to a 'shell' leaving the inner part unlabeled.

In the area postrema tenascin-R (Fig. 3d) had an intense immunoreactivity interrupted by 'holes'. These 'holes' consisted of a transparent 'core' and a rim of the very faint tone of unstained tissues. A dense labeling occurred at the ventrolateral border zone of the organ which seems to be in the position of the funiculus separans. The immunostaining of versican was less intense and less even than that of tenascin-R but delineated a sharp and narrow ventrolateral border zone (not shown in figure). Brevican (Fig. 3e) and neurocan (not shown) had less intense immunoreactivity when compared to the previous ones. Actually, their immunoreactivity hardly surpassed that of the surrounding brain substance but the territory of the area postrema was marked by an intensely stained border zone. Within the organ holes similar to those seen with tenascin-R were faintly recognizable. When WFA was applied the territory of the area postrema displayed intense labeling (Fig. 3f). Ventrolaterally on both sides there was a border zone which had even more intense immunoreactivity than the inner part of the organ.

Of the other organs the OVLT displayed weak immunoreactivity to either versican, or brevican, or neurocan, or tenascin-R and