

Synantocytes: the fifth type of glia? In comparison with astrocytes

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Abstract: To date four types of glial cells have been identified in central nervous system: astrocytes, oligodendrocytes, microglia, ependymocytes. The latest results indicate the existence of the fifth glial type-synantocytes from the Greek word *synanto* that is for contact. Synantocyte processes reach neurons, astrocytes, oligodendrocytes, microglia, synapses, myelin sheaths and nervous fibres' nodes of Ranvier. Morphologically, synantocytes are similar to astrocytes, but they do not contain, like astrocytes, glial fibrillary acidic protein and S-100 β protein. Synantocytes show expression of NG2 surface chondroitin sulphate proteoglycan. Moreover, these cells in contrast to astrocytes do not have membrane transporters for glutamate (Glu), but have receptors for Glu and γ -aminobutyric acid, whose activation can contribute to keeping up ion balance in CNS. Synantocytes are components of synapses, participate in neuronal cytoskeleton stabilization and control myelin integrity, mediate oligodendrocytes answer to nervous fibres' damage and form glial scars. Thus, there are evidences that synantocytes and astrocytes make separate glial population, playing important functions in neuroprotection.

Key words: synantocytes, astrocytes, NG2-glia, NG2

Introduction

For the last 20 years quite intensive examination on morphology and physiology of glial tissue has been carried out. It makes about 90% of central nervous system's (CNS) cells and is crucial for proper function of neurons. The obtained results indicate the existence of a certain group of glial cells which are difficult to classify as the already known 4 glial types i.e. astrocytes (macroglia), oligodendrocytes, microglia and ependyma [1-3]. They were found in both adult and developing hippocampal structures, cerebellum, brain stem and white matter of callosum, as well as in optic nerve. Some researchers described them as immature astrocytes, others as oligodendrocyte progenitor cell (OPCs) and even as cells of all glial types. The lack of agreement proves indefinite character of these cells regarding their identification and performed function in CNS [2,4].

These cells are morphologically similar to astrocytes and they have stellate shape and processes, but unlike them they do not show the presence of characteristic for macroglia, glial fibrillary acidic protein

marker (GFAP) building intermediate glial filaments. Moreover, they do not form among themselves gap junctions, which are commonly present in astroglial net. However, they have on their surface, NG2 immunoreactive chondroitin sulphate proteoglycan and α – receptors for platelet derived growth factor which have been so far recognizable in CNSs [1,2,4]. On this basis, the assumption of the fifth group of glial cells' existence in CNS has come into being [5]. Regarding numerous junctions of these cells with neurons, astrocytes, oligodendrocytes and myelin sheath being their product, as well as nervous fibres in nodes of Ranvier, and microglia, the name synantocytes or synantoglia was suggested from the Greek word *synanto* i.e. for contact [4,6]. These cells were identified with anti-NG2 antibodies, aimed against proteoglycan on their surface. Similar morphology as well as astrocyte and synantocyte distribution do not explicitly let separate these cell groups from each other. Synantocytes are of stellate shape, whereas irregular surface outlines and lack of intermediate glial filaments make them like protoplasmatic astrocytes, while the length and branching model of their processes and dominant existence in white matter are more typical of fibrous astrocytes [7]. It is not definitely clear if synantoglia and astroglia are two different glial populations or one but differentiated. The

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aim of the present paper was to show similarities and differences between these cells on both morphological and functional surface.

Morphological identification of astrocytes and synantocytes.

Astrocytes make about 50% of glial population, occupying about $\frac{1}{4}$ of brain volume and synantocytes about 8-9% in white matter and 2-5% in grey matter [8,9]. Both types of glial cells show similar stellate shapes, with multiprocesses and, probably because of that, they were mistaken for astrocytes. However, the above mentioned similarity is seeming. Thorough examinations showed that these cells differ in regards to their process branching and structure [1].

Thick processes branch off asymmetrically from astrocyte bodies and give numerous secondary branchings, in this way causing branching appearance of these cells. Processes' endings are bulbously expanded and they join together, forming spatial net in meshes of which there are remaining cellular elements of CNS [1]. Through intercellular gap junctions astrocytes form glial syncytium [10]. Their processes form glial limiting membrane: perivascular in shape of end-feet on the surface of capillary vessels, external under pia matter and periventricular – under ependyma of ventricular system [3]. Astrocytic processes reach neurons, what is more they limit synapses and also lie by myelin fibres' nodes of Ranvier [8].

In regards to the character of processes one can distinguish the following astrocytes: protoplasmatic located mainly in grey matter and fibrous occurring mainly in white matter of CNS. Protoplasmatic astrocytes are a little bigger and their numerous relatively short processes branch off plentifully at an obtuse angle not far off the cell body. Fibrous astrocytes give fewer processes which are longer, thinner with numerous secondary branchings splitting at an acute angle far from the cell body [3]. As the examination results revealed plasmatic astrocytes can turn into fibrous astrocytes [11]. In *in vitro* cultures the described type 1 and 2 astrocytes correspond to protoplasmatic and fibrous astrocytes [12]. Astrocytes contain big, oval cell nuclei with very little amount of heterochromatin slightly concentrated under nuclear envelope. The cytoplasm of these cells is bright and poor in cell organelles with a presence of delicate intermediate glial filaments, which are main characteristic feature of fibrous astrocytes. One thinks that protoplasmatic astrocytes can not contain intermediate glial filaments or contain their small amount [3,7]. Cytoplasmatic gliofilaments are made up of glial fibrillary acidic protein (GFAP) – an astrocytic marker, which can be identified with monoclonal antibodies [1,3,6,11]. GFAP distribution in undamaged brain is not equal and con-

stant, but changes dynamically according to prevailing environmental conditions. Astrocyte processes forming end-feet and external glial limiting membrane are intensively GFAP immunoreactive, whereas processes surrounding synapses or nodes of Ranvier do not, in principle, show expression of this protein [1]. Astrocytes except GFAP contain S-100 β protein in nucleus and cytoplasm [13].

Extended cell body, from which a few long and thin processes branching off several times split, are characteristic feature of synantocytes [6,14]. Their end processes narrowing gradually do not form glial limiting membrane just as astrocytes [1]. In grey matter synantocytic processes branching off symmetrically and radially from body cell form circle area of even 200 μm diameter [6,14]. They end by neurons whose cell body they often surround like capsule [4]. Moreover, they can occur by synapses becoming integral component, taking part in synaptic signalization [4,6,14]. In hippocampus synantocyte contacts fewer than 20 synapses on average, in comparison with astrocyte which forms about 100.000 of them [15-17]. Under electron microscope one observed that axon buttons containing fewer synaptic vesicles form junctions with synantocyte processes in which there is a lack of postsynaptic density [2]. In white matter spatial system of a single synantocyte's processes is ellipsoidal. From both poles of extended cell body from 1-3 processes split, which branch off at the length of about 160-200 μm . Their thin branchings contact non-specifically myelin sheath and nervous fibres' nodes of Ranvier [14]. Synantocyte processes also reach other elements of neuropile i.e. cell bodies and astrocyte processes, oligodendrocytes and microglia [6]. Under electron microscope synantocytes resemble astrocytes because they have bright cell nucleus with thin heterochromatin rim under nuclear envelope and visible nucleolus [18]. In thin rim of bright cytoplasm, by poles of extended cell body there are few scattered cell organelles [14]. Mitochondria in comparison with astrocytes are thinner and, among other cell elements, one can come across centrioles more often. Intermediate glial filaments as well as GFAP expression can not be observed in their ultrastructure [18]. Synantocytes do not also contain S-100 β protein. Contrary to astrocytes, they show expression of NG2 proteoglycan made up of chondroitin sulphate which is a membrane protein evenly located on the surface of the whole cell [1,19,20].

Maintaining potassium homeostasis

In CNS glial cells have to form microenvironment appropriate for neurons. During nervous conduction, changes in pH and extracellular space take place. In regulation of ion environment astrocytes play a crucial

role, which, through membrane ion channels, take part in maintaining homeostasis of potassium ions among others [2]. Physiological stimulation of nervous cells causes an increase of potassium concentration in extracellular space from 3 to about 4 mM [10], what, in a effect, activates inwardly K^+ currents in astrocytes through inward rectifying K^+ channels. These ions can be later transported into other areas of CNS through spatial buffer mechanism i.e. astrocytic gap junctions [19,21].

Synantocytes take part in maintaining potassium homeostasis to a smaller extent than astrocytes. Their cell membrane is characterized by weak permeability for ions, thus has high input resistance-above 200 M Ω (between 200-400 M Ω), with negative resting potential measured around 70 mV (white matter) to 90 mV (grey matter), unlike astrocyte resting potential, whose value is 10 M Ω [2,6]. The majority of synantocytes in comparison with astrocytes show smaller inwardly K^+ current, resulting mainly from activation of ATP sensitive K^+ channels stimulated by an increase of extracellular K^+ ions concentration. On the contrary, gap junctions did not develop in synantoglia and cellular outflow of potassium ions takes place through, present in synantocytic cell membranes, quick A-type K^+ channels and slow-delayed outward rectifying K^+ channels.

Involvement of glutamate and γ -aminobutyric acid into homeostasis regulation.

Glial cells have receptors for nearly all neurotransmitters on their surface. A type of receptor and their amount depends on brain area in which a given neurotransmitter dominates. In cell membranes of astrocytes one showed expression of ionotropic and metabotropic receptors for glutamate (Glu) and γ -aminobutyric acid (GABA) [21,22].

Glu as a stimulating neurotransmitter occurs in the majority of CNS regions in mammals. Its extracellular concentration is 2-5 μ M, intracellular- from 3 to 10 mM, while in synaptic vesicles it can reach even 100 mM [10]. In astrocytes one described receptors for Glu (GluR) of ionotropic type: NMDA, AMPA (GluR4) and kainiate as well as metabotropic (mGluR), both groups I (mGluR1 and mGluR5) and groups II (mGluR3) [22]. Depolarization of neuronal presynaptic membrane causes a release of Glu stored in synaptic vesicles which binds with receptors of postsynaptic membrane. Unbound part is instantly picked up into presynaptic part of neurons, but more intensively through astrocytic membrane transporters of excitatory amino acids EAAT-1 and EAAT-2 [10]. In astrocytes the picked up Glu is metabolized by means of glutamine synthetase, to glutamine, which is not a transmitter and it goes to extracellular space and then

to presynaptic neuron. In addition, a big part of Glu taken by astrocytes is used up as metabolic substrate for energy production [1,19,21].

Synantocytes, in comparison with macroglial cells, have only two types of ionotropic receptors for Glu: AMPA (mainly GluR2 and GluR4) and kainiate. Expression of NMDA receptor, as well as mGluR was not observed in these cells [2,4]. In synantocytic cell membranes there is also a lack of glutamate transporters, moreover in cytoplasm there is a lack of glutamine synthetase, what points to their insignificant participation in detecting and deleting of this neurotransmitter in synaptic space [1,19].

Astrocytes also have membrane receptors for GABA – ionotropic GABA_A and metabotropic GABA_B. The presence of these two types of astrocytic receptors was described both *in vivo* as well as *in vitro* [23]. After neuronal stimulation GABA is collected from synaptic space by astrocytes, where it is used for Glu and glutamine production [21].

In synantocytes one showed the presence of GABA_A receptors, with a use of agonist – muscimol, which was revealed by an increase in chloride ion inflow into these cells. It was instantly blocked by gabazine which is these receptors' antagonist. However, the expression of metabotropic receptors GABA_B is unknown [2,4,6]. The above data indicate that synantocytes just like astrocytes are under the influence of both Glu and GABA. However, on the contrary they do not take part in metabolism of these neurotransmitters, but they can be rather involved in maintaining ion homeostasis and pH of extracellular space.

Synantocyte interactions with elements of CNS

Synantocytes interact with the components occurring in extracellular space and membrane particles expressed in synapses, nodes of Ranvier and myelin [6,19]. Active synantocytes produce *e.g.* hyaluronate, versican, phosphacan, neurocan or tenascin influencing neurons. One thinks that substances produced by synantocytes stabilize neuronal cytoskeleton of synapses anchored in ion channels and receptors for neurotransmitters. In this way, these glial cells can have an influence on improving synapse functions and, in the development of CNS, also on synaptogenesis [6]. Similar reactions were observed in nodes of Ranvier, where tenascin and phosphacan produced by synantocytes, bind with B2 subunit of sodium channels numerously occurring in these places [6,19]. Most likely, particles formed by synantocytes interact with various transmembrane proteins of neurons as well as astrocytes and oligodendrocytes. It is suggested that NG2 synantocytic proteoglycan as well as other proteins take part in integrity control mechanism of

myelin and mediate oligodendrocyte answer to damage and degeneration of nervous fibres/myelin sheath [6].

Synantocytes are a population of glial cells instantly responding to nervous tissue's damage. They respond with quick local gliosis and, a signal for that, is continuous ion calcium inflow as an effect of AMPA receptors' activation with high Glu concentration e.g. during hypoxia and ischaemia of CNS [6]. One of the most important functions of synantoglia is its participation in forming glial scars together with astrocytes and microglia. Synantocytes are likely to initiate gliosis when continuity of glial membranes and blood vessels is disturbed. Glial scar formation is strictly connected with, present on their surface, NG2 proteoglycan which interacts with various components among others: plasminogen, collagen (types II, V, VI) or laminine [6,19,20,24]. One described high affinity of NG2 for two growth factors: basic fibroblast growth factor (bFGF) and platelet derived growth factor α (PDGF- α) produced during CNS damage. It is assumed that NG2 proteoglycan can intensify activity of these growth factors on another type of glial cells [24]. However, it is not known if synantocytes, just like astrocytes, produce these or other growth factors.

Synantocytes apart from co-creating protective glial scars probably provide signals for regeneration of damaged axons. However, their role in restitution of the function of damaged nerve tissue is of double type. On the one hand, it seems that they favour reconstruction of damaged nerve connections, on the other they disable this phenomenon [6].

Summary

Neurons and glial cells make interdependent elements of CNS. The astrocytes described in this paper are defined as cells containing GFAP, whereas synantocytes as glial cells containing NG2 proteoglycan on their surface, whose expression was not observed in macroglial cells. There are two models describing interrelation of astrocytes and synantocytes. The former assumes that these cells make the same type of glia, but of a different phenotype, the latter on the other hand, assumes that astrocytes and synantocytes are two separate populations of glial cells [1]. The latter proves the majority of the evidence available. Immunohistochemical examination revealed that cells showing expression of NG2 and GFAP are antigenically different. They also differ as to phenotype, that is why, a range of their function can be different. Astrocytes, unlike synantocytes, produce glial fibres and, in this way, they take part in forming glial limiting membranes as well as blood-brain barrier. Moreover, through presence of stronger inwardly K^+ currents they play an important role in maintaining homeostasis of potassium ions. They also have glutamate mem-

brane transporters thanks to which they trap glutamate released in synapses, limiting in this way its neurotoxic activity. In addition, synantocytes, unlike astrocytes, do not produce glial syncytium by gap junctions, which enable unrestrained ion flow as well as intercellular communication.

To conclude, the hypothesis presenting dissimilarity and recognizing astrocytes and synantocytes as two different populations of CNS glial cells, which play a crucial role in neuroprotection, is highly probable.

Further investigations are necessary to accurately whether synantocytes are the fifth type of glia of CNS in mammals.

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