The role of glial cells in epilepsy

BACKGROUND Brain research in the last century was mainly directed at neurons, with the role of glia assumed to be limited to repair, supplying nutrients and above all simply filling the space between neurons. In recent years, the importance of glial cells for normal brain function has been recognised. This article summarises knowledge of glial cells relevant to epilepsy.

METHOD The article is based on a literature search in PubMed as well as the authors' clinical and research experience.

RESULTS Astrocytes are the largest subgroup of glial cells and, in common with neurons, express a diversity of membrane transporters, ion channels and receptors. Among the most important roles of astrocytes are the uptake and redistribution of ions and water, glucose metabolism and communication with nerve cells. Disturbances in all of these functions have been associated with epilepsy.

INTERPRETATION Epilepsy has previously been regarded as almost exclusively a disturbance in the functioning of neurons and especially of their contact points, the synapses. The mechanisms of action of today's anti-epileptic drugs are therefore primarily directed at neuronal channels and receptors. New knowledge of the role played by glial cells could increase our understanding of how epilepsy arises and lead to new treatment strategies.

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MAIN POINTS

Glial cells are the largest group of cells in the brain and make up roughly half of its volume.

Interactions between glial cells and neurons are crucial for normal brain function.

Glial cell dysfunction most likely can contribute to or even cause epilepsy.

New knowledge on the role of glial cells in the brain can open up new therapeutic possibilities for epilepsy.

Epilepsy is perhaps the most complex brain disorder. Despite increasing knowledge of the condition and the availability of a multitude of drugs, one third of patients experience repeated and debilitating seizures (1). Currently, almost all anti-epileptic drugs are targeted at *neurons*, mainly at channels and receptors within the cell membrane. However, new knowledge about glial cells (from the Greek gloios; sticky substance) has led to an increased understanding of epilepsy and has opened the door to entirely new treatment strategies.

This article summarises current knowledge of glial cells and outlines potential targets for future epilepsy treatments.

Method

This review is based on our own research and on a search in PubMed using the keywords «epilepsy AND astrocyte» through to the end of 2012 (1,089 hits). Only English-language articles were included. A discretionary selection of the most relevant original articles and reviews was made based on knowledge of the international academic community and of the most significant journals within the field.

Astrocyte dysfunction

New research has shown that the largest subgroup of glial cells – astrocytes – in common with neurons express a diversity of membrane proteins and channels. There is now a range of evidence that astrocytes are able to «monitor» synaptic activity, encode and integrate synaptic information and release signalling molecules that can alter neuronal activity. The following section describes the most important properties of astrocytes, as well as how disturbances in these can contribute to or cause epilepsy.

Water and potassium balance

Two key channel proteins in the astrocyte membrane are responsible for maintaining water and potassium balance in the brain: the potassium channel Kir4.1 and the water channel aquaporin-4 (AQP4) (Fig. 1a).

Potassium channel Kir4.1

Neurons constantly release potassium, including in conjunction with neurotransmission at synapses. This leads to an increase in the potassium concentration in the extracellular space around the synapses. Potassium must be quickly and efficiently removed from these areas of high concentration as it could otherwise depolarise nearby neurons and thereby precipitate epileptic seizures. Membrane proteins such as the Na-K-pump and various K-transporters deal with potassium uptake. Astrocytes also use these to take up potassium, but the most important channel in this regard most probably is Kir4.1 (potassium inwardly rectifying) (Fig. 2). A number of studies have confirmed that Kir4.1 dysfunction increases the propensity for epileptic seizures. The phenotype of the Kir4.1-knockout mouse is characterised by severe epilepsy that often leads to death a few weeks postnatally (2). Kir4.1 deficiency and impaired potassium uptake in the hippocampus have been reported in patients with mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) (3–6). In addi-

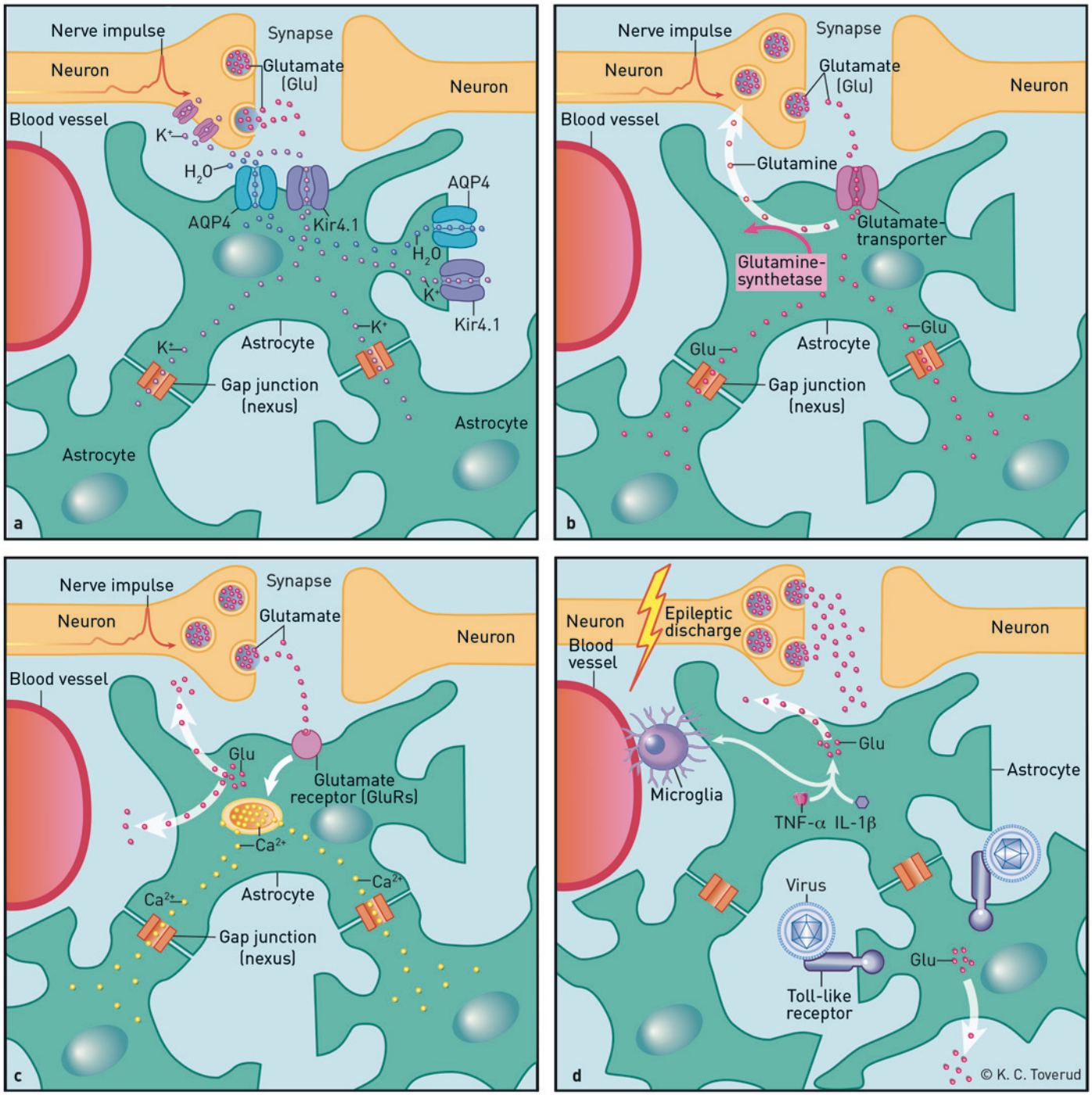

Figure 1 *Astrocyte functions relevant to epilepsy. a) Potassium and water balance. Potassium is released during synaptic transmission and is taken up by astrocytes through the potassium channel Kir4.1. This is assumed to occur together with uptake of water through the water channel AQP4. Potassium can be redistributed further to neighbouring astrocytes via gap junctions and can be released into the perivascular extracellular space through channels in the astrocyte end-feet. b) Glutamate uptake and metabolism. Glutamate released during synaptic activity is taken up via glutamate transporters located in the astrocyte membrane. Inside the astrocytes, glutamate is converted to glutamine with the help of the enzyme glutamine synthetase, before being taken back up into the presynaptic terminal. In common with potassium and other ions, glutamate probably can also be redistributed via gap junctions. c) Gliotransmission and calcium signalling. Activation of glutamate receptors (GluR) in the astrocyte membrane triggers Ca^{2+} release from intracellular stores. An increase in intracellular Ca^{2+} concentration is assumed to trigger secretion of transmitter [glutamate (Glu)] by the astrocytes (gliotransmission). d) Immune response. Astrocytes are implicated in a number of immunological processes in epilepsy. Shown here is the release of the cytokines IL-1β and TNF-α from astrocytes as an inflammatory response to neuronal epileptic discharges. IL-1β and TNF-α induce glutamate release and the migration of microglia, which in turn contribute to inflammation. Astrocytes possess toll-like receptors that can be activated by microbes, which could lead to glutamate secretion*

tion, mutations in the gene encoding Kir4.1 have been described in a number of families with EAST syndrome, in which epilepsy is one of the main symptoms (7, 8).

Thus, there is a range of evidence that Kir4.1 dysfunction can play a role in epilepsy.

Water channel AQP4

A number of studies suggest that Kir4.1 is co-localised with the main water channel in the brain, AQP4 (9, 10). Both channels are present at particularly high concentrations in the so-called end-feet of astrocytes, which encircle the brain's blood vessels and form the outermost layer of brain tissue (11, 12). That the channels are co-localised in astrocytes supports the theory that potassium uptake is linked to water uptake; that is, that the channels are functionally connected (13).

Several studies have associated AQP4 dysfunction with epilepsy. Mice that lack the gene for AQP4 (AQP4 «knockout») do not suffer from epilepsy, but they experience longer-lasting epileptic seizures upon electrical stimulation of the cortex than do non-genetically modified mice (14). Mice that lack the molecule that anchors AQP4 in the cell membrane, alpha-syntrophin, show a reduced ability to take up potassium from the extracellular space (15). Brain MRI in patients with MTLE-HS often shows increased water content in the sclerotic hippocampus, which can indicate a localised disturbance in the water balance (16). Electron microscopy has shown reduced perivascular AQP4 in patients with this form of epilepsy (17).

Gap junction network

In contrast to neurons, astrocytes are connected in a network via gap junctions (Fig. 1) (18). These structures create direct connections between the cytoplasm of cells of the same type, which means that different molecules and ions can pass directly from cell to cell. A range of ions, amino acids and other substrates, including K^+, glutamate, adenosine triphosphate (ATP), Ca^{2+} and inositol trisphosphate (IP_3), can pass via these channels from astrocyte to astrocyte (19). This enables potassium, for example, to be rapidly redistributed from areas of high to low concentration. Likewise Ca^{2+}, which is an important substrate for intracellular signalling, can be rapidly redistributed via gap junctions and may contribute to «information flow» from astrocyte to astrocyte. Referred to as Ca^{2+}-signalling, this principle will be discussed further in a subsequent section. Connections between astrocytes allow rapid redistribution of excess transmitters and ions. New research suggests that disruption of these connections may play a role in epilepsy (20).

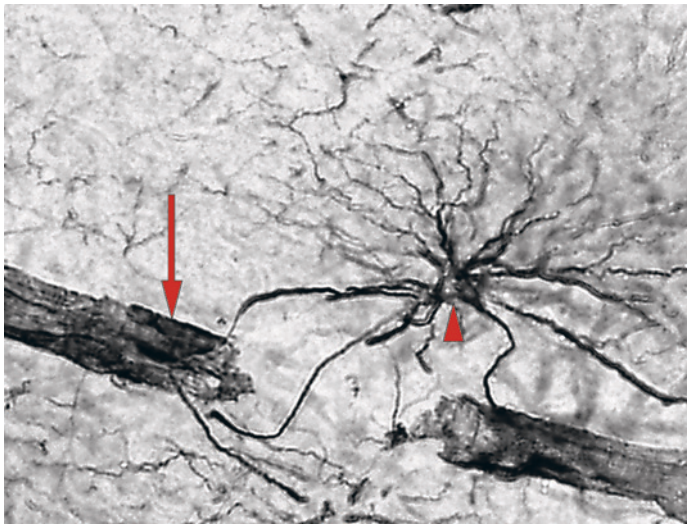

Figure 2 *Astrocyte extending processes towards the brain parenchyma and blood vessels. Immunohistochemical visualisation of the potassium channel Kir4.1 in the human hippocampus. Astrocyte with processes (arrow head) and end-feet covering the surface of a blood vessel (arrow)*

Glutamate metabolism

The amino acid glutamate is the predominant excitatory neurotransmitter in the nervous system. Because of its strong excitatory – and at high concentrations cytotoxic – effect, it is important that glutamate is quickly inactivated after its release into the synaptic cleft. High glutamate concentrations would otherwise lead to increased neuronal excitation and probably to epilepsy (21). Astrocytes are responsible for uptake and conversion of glutamate released during synaptic activity (Fig. 1b). This is achieved via glutamate transporters in the astrocyte membrane. After uptake by astrocytes, glutamate is converted into glutamine with the aid of the enzyme glutamine synthetase. Disturbances in astrocyte-mediated glutamate metabolism have been associated with epilepsy. A reduction in glutamine synthetase was found in the epileptogenic sclerotic hippocampus of patients with MTLE-HS, for example (22).

Glutamate is most readily taken up by astrocytes at a membrane potential close to the equilibrium potential of potassium (23, 24). Kir4.1 dysfunction (discussed above) can thus lead to suboptimal glutamate uptake, and increase the propensity for epileptic seizures.

Gliotransmission and calcium signalling

Gliotransmission is a relatively new and still somewhat controversial concept that denotes the ability of astrocytes to release chemical transmitter substances (Fig. 1c). The novelty lies in the idea that not only can neurons send signals to each other, but astrocytes too can send chemical signals to neurons through secretion of transmitter substances. An important study in this respect was published in 1994 in *Nature* (25). The authors studied astrocytes in cell culture and showed that they could release glutamate, a property that until then was assumed to be limited to neurons. This study has since been followed by a number of publications on interactions between astrocytes and neurons (26, 27). Glutamate release from astrocytes is assumed to occur via an increase in the intracellular calcium concentration (28, 29). Several studies, including our own unpublished data, show that electrical stimulation of neurons leads to calcium release in a network of astrocytes. However, the function of these calcium signals is still unclear. Increased glutamate receptor expression in astrocytes has been reported in animal models of temporal lobe epilepsy (30, 31). Increased stimulation of astrocytes via these receptors could conceivably lead to increased astrocytic glutamate release, which could in turn lead to neuronal excitation and give rise to epilepsy.

Immune response and inflammation

Epileptic seizures are known to be associated with a range of immunological processes and with inflammation in the brain (32). Astrocytes contribute to these processes largely via the production of inflammatory substances such as chemokines and cytokines, particularly interleukin-1-beta (IL-1β) and tumour necrosis factor-alpha (TNF-α), and release these in association with epileptic activity (Fig. 1d) (32, 33). Moreover, inflammation can lead to changes in the expression of genes encoding various membrane proteins (channels, transporters

and receptors) in astrocytes (33). Conformation changes of these can, in turn, enhance epileptic activity via the mechanisms outlined above.

Brain infections, especially bacterial abscesses and viral infections, are often associated with febrile seizures in childhood or with epileptic seizures. Here, too, it is probably the inflammatory process itself that increases the propensity for epileptic seizures rather than the causative agents *per se* (33).

Astrocytes express so-called toll-like receptors (TLR) that recognise and react to microbes (34, 35). Activation of these receptors can lead to stimulation of the transcription factor NFκB (nuclear factor «kappa-light-chain-enhancer» of activated B-cells), which can, in turn, increase astrocytic glutamate release (36).

Complex febrile seizures in children predispose to the development of temporal lobe epilepsy, and febrile seizures are associated with increased IL-1β levels in the central nervous system and serum (37). IL-1β increases excitability by enhancing glutamate receptor function and by inhibiting GABAergic transmission (32). In vitro experiments have shown that IL-1β and TNF-α act on astrocyte receptors to inhibit glutamate uptake and increase its secretion (38). During status epilepticus, activated microglia release IL-1β and TNF-α, which disrupt communication between astrocytes by downregulating gap junction proteins (39).

Astrocytes are thus involved in a number of inflammatory processes that can contribute to or trigger epileptic seizures.

Conclusion

Until recently, epilepsy was regarded as a condition almost exclusively related to neurons. It is only in the last few years that epilepsy has also been associated with dysfunctions of glial cells, particularly astrocytes. Astrocytes are essential for maintaining the brain's water and ion balance. Astrocytes interact closely with neurons and modulate synaptic transmission by signalling back to neurons. In addition, astrocytes are involved in inflammatory processes and contribute to the immune response. Moreover, astrocytes have important homeostatic functions such as uptake and conversion of the excitatory neurotransmitter glutamate. A number of studies, including animal models and studies of human brain tissue, have shown pathological changes in astrocyte function in epilepsy. Thus, it is highly likely that disease processes in astrocytes contribute to or are even a direct cause of epilepsy. Our knowledge of astrocyte function and of the interactions between astrocytes and neurons is still limited. However, research within this field has the potential to rapidly acquire great clinical significance by opening up entirely new therapeutic possibilities for epilepsy.

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