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MINIREVIEW

Pathological Potential of Astroglia

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Summary

The pathological potential of glial cells was recognized already by Rudolf Virchow, Santiago Ramon y Cajal and Pio Del Rio-Ortega. Many functions and roles performed by astroglia in the healthy brain determine their involvement in brain diseases; as indeed any kind of brain insult does affect astrocytes, and their performance in pathological conditions, to a very large extent, determines the survival of the brain parenchyma, the degree of damage and neurological defect. Astrocytes being in general responsible for overall brain homeostasis are involved in virtually every form of brain pathology. Here we provide an overview of recent developments in identifying the role and mechanisms of the pathological potential of astroglia.

Key words

Astrocyte • Astrogliosis • Brain pathology • Brain damage and repair • Ischemia • Acute brain trauma • Neurodegeneration

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Introduction

Astroglial cells form a true backbone of the grey matter, by shaping the micro-architecture of the brain, creating independent neuronal-glial-vascular units (Fig. 1), providing neurons with energy and controlling extracellular ion- metabolite- and neurotransmitter homeostasis (Araque *et al.* 1999, Nedergaard *et al.* 2003, Zonta *et al.* 2003, Haydon and Carmignoto 2006, Magistretti 2006, Oberheim *et al.* 2006, Verkhratsky 2006b,a, Verkhratsky and Toescu 2006, Giaume *et al.* 2007, Verkhratsky and Butt 2007). Further, astroglia actively participate in information transfer by accepting incoming information through a multitude of neurotransmitter receptors residing in astrocyte membranes (Verkhratsky *et al.* 1998, Verkhratsky and Steinhauser 2000, Volterra and Meldolesi 2005, Lalo *et al.* 2006, Verkhratsky and Kirchhoff 2007a,b) and feeding information back by virtue of regulated gliotransmitter release (Bezzi *et al.* 2004, Volterra and Meldolesi 2005). Information processing within astroglial networks, created by gap junctions connecting the terminal processes of astrocytes, operates in an intercellular volume transfer mode (Dermietzel 1998, Scemes and Giaume 2006) by direct exchange of second messengers, metabolites and other yet unidentified signalling molecules. This specific signaling mode brings much sophistication to information transfer and can potentially be relevant for higher brain functions (Allen and Barres 2005, Verkhratsky and Butt 2007). Finally, astrocytes control the genesis, survival and death of synapses (Ullian *et al.* 2004) and support adult neurogenesis through "stem" astrocytes subpopulations (Barres 1999, Berninger *et al.* 2006).

The pathological potential of glial cells was recognized already by Rudolf Wirchov (Tower 1992). Many functions and roles performed by astroglia in the healthy brain determine their involvement in brain diseases; as indeed any kind of brain insult does affect

astrocytes, and their performance in pathological conditions, to a very large extent, determines the survival of the brain parenchyma, the degree of damage and neurological defect (Fig. 2). Furthermore, brain insults trigger a specific astroglial reaction, generally known as reactive astrogliosis. Astrogliosis is a defensive reaction, which is instrumental for limiting the areas of brain damage (by forming a scar) and for the aftermath of the lesions when reactive astroglia assists in the remodeling of the neural circuitry (Pěkný and Nilsson 2005).

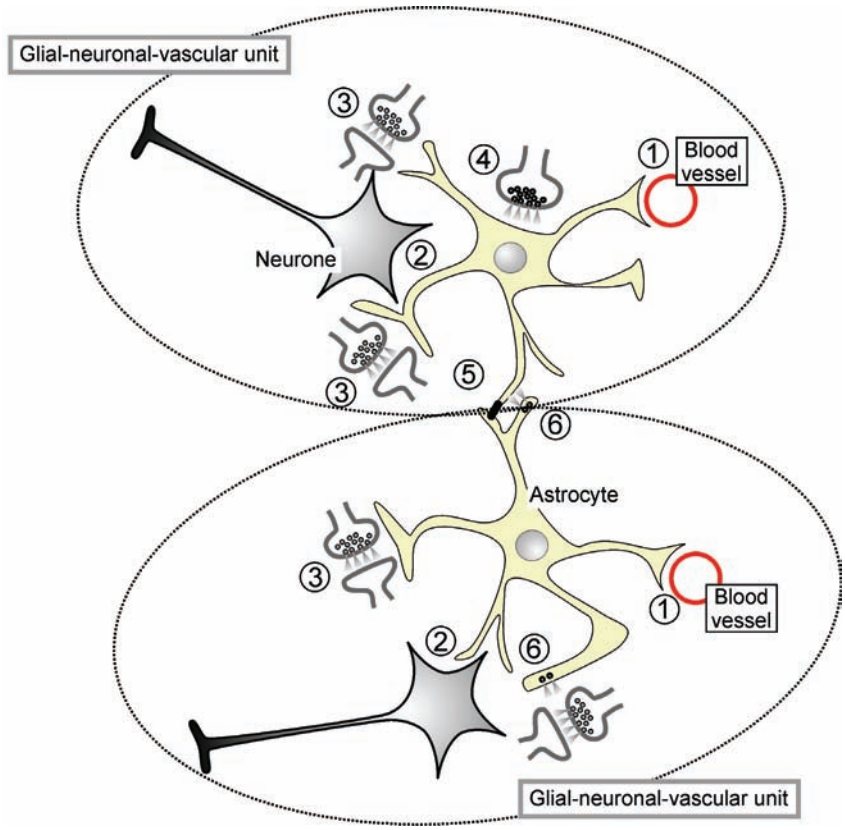

Fig. 1. Integrative role of astroglia. Grey matter astrocytes occupy clearly defined territories, where they form contacts with all neuronal membranes and send endfeet to neighboring capillaries thus creating relatively independent glial-neuronal-vascular units. (1) The astroglial endfeet plaster the outer capillary wall and secrete yet unknown factors, which determine the appearance of tight junctions and hence the formation of the blood-brain barrier. By virtue of numerous transporters, astroglial cells provide for metabolite exchange between the brain parenchyma and blood vessels. Astroglial cells also release vasoconstricting/ vasodilating agents, which couple neuronal activity with local blood flow. (2) Astrocytes provide active neurons with energy substrates using the "astrocyte-neuronal lactate shuttle". (3 – 4) Astrocytes receive signaling input from neurons using a host of receptors residing in astroglial membranes forming a "tripartite synapse" or in direct neuronal-glial synaptic contacts. (5) Astrocytes communicate between themselves by gap junctions or through the release of gliotransmitters. (6) Astrocytes communicate with neuronal circuits by gliotransmitters, which modulate synaptic transmission and affect neuronal excitability.

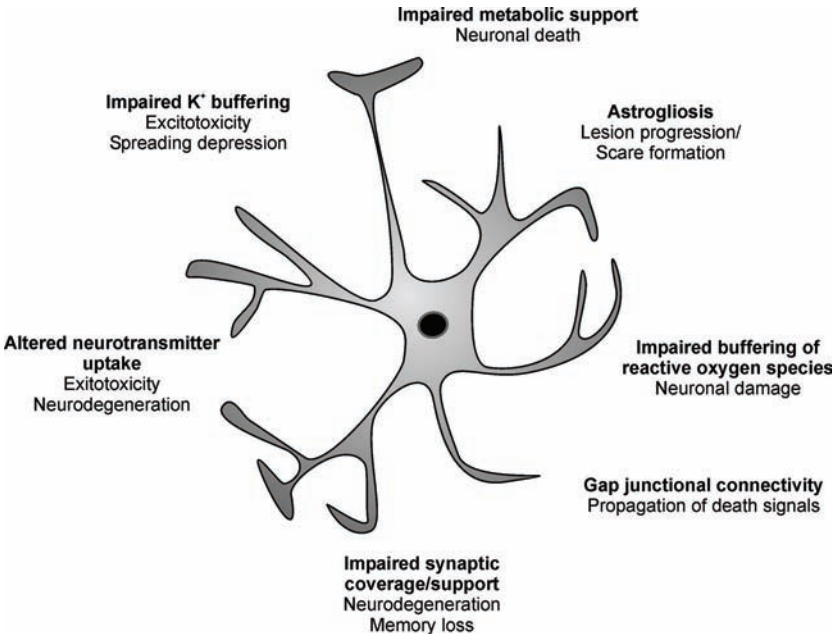

Fig. 2. Pathological potential of astrocytes.

Astroglia and ischemic insults

As very often happens in nature, astroglia play a dual role in brain insults, providing for both defense and destruction of neural tissue (Nedergaard and Dirnagl 2005, Rossi *et al.* 2007). Indeed, incidences of reduced oxygen supply immediately trigger neuronal depolarization, with subsequent loss of ion homeostasis. Depolarization-induced Ca^{2+} influx results in massive release of glutamate, which induces further depolarization thus causing glutamate excitotoxicity, which assumes the central role in brain damage (Westbrook 1993). Over-activation of glutamate receptors results in massive cell overload with Ca^{2+}, which in turn launches multiple forms of cell death (Nicotera *et al.* 2007).

Astrocytes represent the main part of the brain's defense against glutamate excitotoxicity, because they express high densities of glutamate transporters and accumulate up to 80 % of total glutamate released in the brain (Danbolt 2001, Kirischuk *et al.* 2007). Moreover, astrocytes express huge amounts of the major ROS scavengers and anti-oxidants glutathione and ascorbate, which protect the brain against oxidative stress accompanying ischemia. Finally, by virtue of both local and spatial K^+ buffering astroglia remove excess K^+ from the extracellular space thus restraining neuronal depolarization (Giaume *et al.* 2007). Yet, in conditions of prolonged ischemia, glutamate cannot be contained within astroglia; depolarization and alteration of sodium gradients result in the reversal of glial glutamate transporters (Allen *et al.* 2004) and further massive release of glutamate; in addition, glutamate can be released from astroglia *via* exocytosis or through large membrane pores formed by, e.g., hemichannels or $P2X_7$ receptors (Contreras *et al.* 2004).

The second important role played by astroglia in the progression of ischemic insults is associated with their intercellular connectivity, which is formed by gap junctions and unifies astrocytes into functionally continuous syncytium (Giaume *et al.* 1991, Bruzzone and Giaume 1999). Once more intercellular volume transmission through gap junctions plays a dual role in responses to ischemic injury as it may participate in either the removal of unwanted substances (e.g., K^+ buffering) or in spreading pathological signals. Both neuroprotective and detrimental roles of the astroglial syncytium received experimental support. Indeed, the neuroprotective role of the astroglial web is corroborated by the following observations: (i) inhibition of astroglial gap junctions by pharmacological agents enhanced neuronal vulnerability (ii) partial genetic deletion of Cx43, which forms a substantial part of glial gap junctions *in vivo*, increased sensitivity of neural tissue to stroke, which was manifested by a significant increase of stroke volume and (iii) specific deletions of Cx43 in astrocytes also increased neuronal vulnerability to ischemia (Giaume *et al.* 2007). At the same time a wealth of data supporting the pathological potential of gap junctional communications has been acquired recently. First, it was shown that astroglial gap junctions remain open during ischemia, and they can propagate certain death signals (Cotrina *et al.* 1998, Lin *et al.* 1998). Second, it appeared that inhibition of Cx43 expression by specific antisense oligodeoxynucleotides reduces neuronal death in response to glucose and oxygen deprivation, and pharmacological blockade of gap junctions decreases the stroke volume following occlusion of the medial cerebral artery (Nedergaard and Dirnagl 2005). Finally, gap junctions may participate in the generation of waves of spreading depression through the penumbra, which are critical for expansion of the infarct zone (Budd and Lipton 1998). It still remains unclear which conditions favor a neuroprotective or detrimental impact of the glial syncytium; they may depend on the severity of insult and brain region.

Astroglia and acute brain trauma

Mechanical brain injury or injections of toxic substances into the brain tissue (e.g. kainate lesions) trigger massive cell death and astrogliosis manifested by elevated expression of glial fibrillary acidic protein, GFAP (Bignami and Dahl 1977, Hozumi *et al.* 1990). The membrane properties of astrocytes in post-traumatic brain or spinal cord were investigated *in vitro*, in cultures of cortical (Perillan *et al.* 1999, Perillan *et al.* 2000) and spinal cord astrocytes (MacFarlane and Sontheimer 1997, 1998). These studies have demonstrated that reactive spinal cord astrocytes up-regulated several membrane conductances, including delayed outwardly rectifying K^+ currents (K_{DR}), transient A-type K^+ currents (K_A) and voltage-gated Na^+ currents. Proliferating astrocytes demonstrated a down-regulation of inwardly rectifying currents (K_{IR}), whereas in non-proliferating astrocytes K_{IR} currents were increased.

Since many pathological states are accompanied by an increase in $[K^+]e$, an early event leading to the activation of astrocytes and the subsequent formation of a glial scar, several studies have examined the astrocyte

membrane properties and cell volume regulation of astrocytes after exposure to high K^+ *in situ* (Anderova *et al.* 2001, Vargová *et al.* 2001, Anderova *et al.* 2004, Neprašová *et al.* 2007). Neprašová *et al.* (2007) found that spinal cord astrocytes, exposed to elevated K^+, reacted by both morphological changes and the alteration of membrane properties and cell volume regulation.

The astrocytic response to a mechanical trauma, such as a cortical stab wound, is manifested by intense immunostaining for GFAP (Enclancher *et al.* 1990, Vijayan *et al.* 1990, Kálmán and Ajtai 2000, Nolte *et al.* 2001), S-100β, a calcium-binding protein that is predominantly found in astrocytes, and for vimentin, a cytoskeletal protein expressed in reactive astrocytes (Perillan *et al.* 1999, Perillan *et al.* 2000). Nestin expression in reactive astrocytes has been also detected (Yagita *et al.* 2002, Anděrová *et al.* 2004). Astrogliosis also increases diffusion barriers in the CNS due to the hypertrophy of astrocytic processes and the increased production of extracellular matrix components (Syková 1997, Roitbak and Syková 1999, Syková and Chvátal 2000). This can impair the diffusion of ions, neurotransmitters, trophic factors and other neuroactive substances in the brain and thus influence the extent of CNS injury.

The voltage-dependent K^+ and Na^+ currents in reactive astrocytes have been extensively studied *in situ* (Jabs *et al.* 1997, D'Ambrosio *et al.* 1999, Bordey *et al.* 2001). These studies have shown that reactive astrocytes express predominantly K_{DR} while the expression of K_{IR} is decreased, which may imply an impaired K^+ spatial buffering capacity and a failure of ionic homeostasis in gliotic CNS tissue, followed by abnormal neuronal activity. Expression of voltage-gated K^+ channels in astrocytes *in vivo* is affected by astrocyte proliferation at the site of injury; similar results were found in *in vitro* models of astrogliosis (MacFarlane and Sontheimer 1997, 1998). That is, proliferating astrocytes (identified by bromo-deoxyuridine staining) in the cortex of young rats (P16 - 24), which received a focal cortical freeze-lesion on the first postnatal day, demonstrated increased expression of K_{DR} channels, but they did not appear to express K_{IR} channels at all (Bordey *et al.* 2001). Investigations of Anderova *et al.* (2004) revealed the existence of two electrophysiologically, immunohisto-chemically and morphologically distinct types of hypertrophied astrocytes at the site of a stab wound, depending on the distance from the lesion (Fig. 3). "Proximal astrocytes", found within a distance of ~100 μm from the stab wound, showed an up-regulation of K_{DR} currents and were nestin and BrdU-positive, while nestin and BrdU-negative astrocytes, showing an up-regulation of K_{IR} currents from 6 hours to 3 days after trauma, were localized more distantly from the site of wound (> 100 μm).

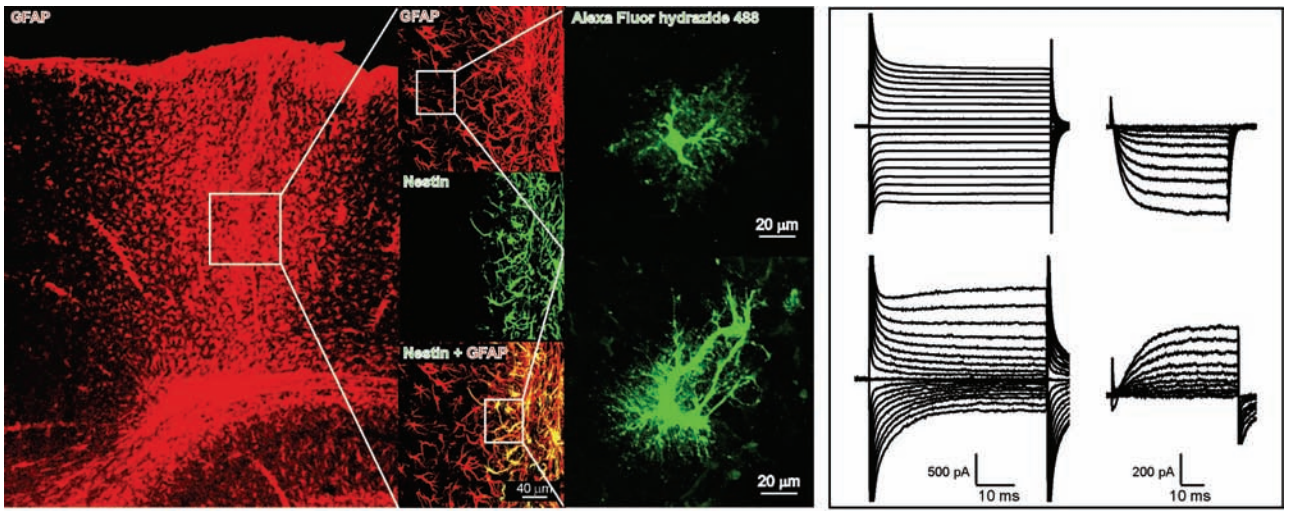

Fig. 3. Two immunohistochemically and electrophysiologically distinct types of reactive astrocytes were detected in the vicinity of a stab wound. Immunohistochemistry of a coronal section from the cortex of an injured rat 7 days post-injury-PI (lower magnification, left). Sections of the brain slices were stained for GFAP. Note the stronger expression of GFAP in the vicinity of the stab wound. Immunostaining for GFAP and nestin (higher magnification) and double immunostaining for nestin and GFAP (bottom, high magnification) in the cortex of a wounded rat 7 days PI. Note the presence of nestin-positive astrocytes (nestin+GFAP) only in the vicinity of the wound (double stained cells are yellow) and astrocytes positive only for GFAP**.** Typical membrane current patterns of astrocytes in the cortex of control rats and 7 days PI (right). Membrane current patterns in GFAP-positive and GFAP/nestin-positive were measured in response to voltage steps from a holding potential of -70 mV. To activate the currents, the membrane was clamped for 50 ms from the holding potential of –70 mV to increasing de- and hyperpolarizing potentials ranging from -160 to +20 mV, in 10 mV increments. Note the large amplitude of inwardly rectifying K^+ currents in GFAP-positive astrocytes and large amplitude of outwardly rectifying K^+ currents in GFAP/nestin-positive astrocytes.

Using several *in vivo* models of chemical CNS injury it was found that changes in the expression of various ion channels in post-traumatic astrocytes are directly affected by both the nature and the extent of the tissue injury. For instance, injection of kainate into the ventricles caused degeneration of hippocampal pyramidal cells in the CA3 region in concert with a significant reduction of functional Ca^{2+} channels in astrocytes in acutely isolated hippocampal slices (Burnard *et al.* 1990). Lesions induced by an intraperitoneal injection of kainate resulted in a loss of tetrodotoxin-sensitive Na^+ channels in reactive astrocytes in the adult rat hippocampus (Jabs *et al.* 1997). In the rat hippocampus, fluid percussion injury induced a decrease of K_A and K_{IR} currents (D'Ambrosio *et al.* 1999). Similarly, K_{IR} currents were reduced in astrocytes from the dentate gyrus of adult rats subjected to an entorhinal cortex lesion (Schroder *et al.* 1999).

Astroglia and chronic neurodegeneration

Post-stroke dementia is a frequent outcome of ischemic insults; neurological defects that develop after stroke to a large extent are determined by the glia, because the degree of astrogliosis and its progression directly influence the size of the infarction and posttraumatic regeneration and remodelling.

Alzheimer's disease (AD), named after Alois Alzheimer who was the first to describe this pathology in 1907 together with post-stroke dementia, is the main cause of senile dementia. Progression of AD is associated with profound neuronal loss throughout the brain which rapidly affects memory and results in severe impairment of cognitive functions. Histological hallmarks of AD are (i) the formation of deposits of β-amyloid protein (Aβ) in the walls of blood vessels; (ii) the accumulation of Aβ plaques in the grey matter and (iii) the intra-neuronal accumulation of abnormal tau-protein filaments in the form of neuronal tangles (Dickson 1997, Selkoe 2001). AD is associated with prominent reactive astrogliosis and the activation of microglia (incidentally, the involvement of glial cells in the pathogenesis of AD was initially suggested by Alois Alzheimer himself in 1910). In fact, AD plaques are formed by Aβ deposits, degenerating neurites, astroglial processes and activated microglial cells (Wisniewski and Wegiel 1991).

Astrocytes appear as natural scavengers of Aβ, and in particular its toxic truncated form Aβ42 (Nagele *et al.* 2003). Astrocytes detect Aβ deposits, cover them with their processes and take up and degrade the Aβ. This ability of astrocytes to take up Aβ allowed Robert Nagele and his co-workers to propose a hypothesis about a leading role of astroglia in the progression of AD (Nagele *et al.* 2004). According to this hypothesis, at the very early stages the initial production of Aβ42 in neurons trigger their initial degeneration and release of Aβ42. The latter, together with products of neuronal destruction, activates neighboring astrocytes, which in turn start to accumulate Aβ and clear the neuronal debris. Indeed, astroglial load by Aβ42 directly correlates with the local density of plaques and the amount of extracellular Aβ42 (Nagele *et al.* 2003). Incidentally, Aβ42 accumulation coincides with significant increase in the concentration of neuronal nicotinic acetylcholine receptors in astrocytes, probably reflecting the very high affinity of the latter for Aβ42 (Nagele *et al.* 2004).

Overload of astrocytes with Aβ42 compromises their function, thus affecting their support of other neurons within the astrocyte domain. Withdrawal of astrocytic support may initiate the degeneration of synapses and trigger the distant accumulation of Aβ42. In addition, astroglia may even be instrumental in Aβ42 neurotoxicity: *in vitro* experiments have shown that treatment of astroglial-neuronal co-cultures with Aβ results in the generation of $[Ca^{2+}]_i$ oscillations in astrocytes, without any apparent $[Ca^{2+}]_i$ changes in neurons (Abramov *et al.* 2004a,b). These astroglial oscillations induced neuronal death in ~24 hours; inhibition of glial $[Ca^{2+}]_i$ responses was neuroprotective (Abramov *et al.* 2004a,b). Degeneration of the whole astrocyte domain results in lysis and the formation of an astroglial plaque. Subsequently, neighboring astrocytes become activated and send their processes towards the plaque, trying to clear the excess Aβ. The repetition of this process eventually recruits increasing numbers of astrocytes and through them astrocytic domains with their neurons, which in turn leads to dissemination of the plaques and neurological defects.

Amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS), also known as 'Lou Gehrig's disease' (named after a baseball player who died from ALS in 1941) was initially described by Charcot in 1869. This disease is manifested by the degeneration of motor neurons located in the cortex, brain stem and spinal cord (Mitchell and Borasio 2007). This neurodegeneration results in progressive paralysis and muscle atrophy. The key pathological determinant of neuronal death in ALS is associated with deficient glutamate clearance, and as a

consequence, excitotoxic neuronal damage. This deficient glutamate clearance results from the disappearance of the astroglial glutamate transporter EAAT2 in the affected brain areas (Barbeito *et al.* 2004) as a consequence of gene failure and may result from aberrant RNA splicing, exon skipping and intron retention. Experimental genetic deletion of EAAT2 (GLT-1) in mice faithfully mimicked ALS and led to the degeneration of motor neurons (Barbeito *et al.* 2004).

Effects of elevated extracellular K^+ concentration on astroglia

Rapid increases in extracellular K^+ concentration ($[K^+]_e$) in the CNS occur under many pathological states, such as ischemia, epileptical seizures, traumatic brain injury or spreading depression. In these pathological conditions $[K^+]_e$ can be elevated up to 80 mM, which significantly contributes to the damage of the nervous tissue (Somjen 1979, Syková 1983, Syková *et al.* 1992, Vorîšek and Syková 1997, Somjen 2001). In addition, elevated $[K^+]_e$ may also trigger cell proliferation (Del Bigio *et al.* 1994) and can induce or modify apoptotic cell death (Yu 2003). Astroglia are responsible for extracellular K^+ homeostasis in the CNS; astrocytes remove excess $[K^+]_e$ through both K^+ uptake by K^+ channels or transporters and through K^+ spatial buffering within the astroglial syncytium (Orkand *et al.* 1966, Somjen 2001, Kofuji and Newman 2004). Acute exposure of astrocytes to elevated $[K^+]_e$ results in reversible membrane depolarization, accumulation of intracellular K^+ and rapid cell swelling (Pasantes Morales and Schousboe 1988, Walz 1997).

Astrocyte swelling, which can be modelled *in vitro* by exposure to hypotonic solution or to an isotonic solution with an increased $[K^+]$ (Kimelberg *et al.* 1995, Chvátal *et al.* 1999, Anděrová *et al.* 2001, Vargová *et al.* 2001), evokes a large increase in extracellular K^+ in the vicinity of the cell membrane after a transient depolarization, the latter resulting from an extracellular space (ECS) volume decrease around swollen astrocytes. Syková *et al.* (1999) found that incubation of the spinal cord in 50 mM K^+ evokes cell swelling resulting in a decrease in the ECS volume fraction and astrocyte activation (manifested by an increase in GFAP immunoreactivity). This can lead to the impairment of both synaptic and extrasynaptic transmission, the diffusion of neuroactive substances and neuron-glia communication in the CNS (Syková 2005).

As has been discussed before, reactive astrocytes change the pattern of K^+ channels by down-regulating the expression of K_{IR} and increasing the expression of K_{DR}. A decrease in K_{IR} may directly impair K^+ buffering capacity and thus result in a failure of ionic homeostasis in gliotic CNS tissue, followed by abnormal neuronal activity. Our recent study (Neprašová *et al.* 2007) demonstrated that in complex astrocytes, pre-incubation with high K^+ caused depolarization, an increase in input resistance, a decrease in membrane capacitance and an increase in the densities of voltage-gated K^+ and Na^+ currents. Conversely, in passive astrocytes the reversal potential shifted to more positive values and the densities of K^+ and Na^+ currents decreased. No changes were observed in astrocyte precursors.

Conclusions

Astrocytes are involved in virtually every type of brain pathology. They play a dual role forming the brain defense system and at the same time exacerbating brain damage when severely insulted. Astroglial performance to a very large extent determines the outcome of brain pathology and the degree of neurological damage.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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