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Astrocyte signaling and neurodegeneration
New insights into CNS disorders

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Growing evidence indicates that astrocytes cannot be just considered as passive supportive cells deputed to preserve neuronal activity and survival, but rather they are involved in a striking number of active functions that are critical to the performance of the central nervous system (CNS). As a consequence, it is becoming more and more evident that the peculiar properties of these cells can actively contribute to the extraordinary functional complexity of the brain and spinal cord.

This new perception of the functioning of the CNS opens up a wide range of new possibilities to interpret various physiological and pathological events, and moves the focus beyond the neuronal compartment toward astrocyte-neuron interactions. With this in mind, here we provide a synopsis of the activities astrocytes perform in normal conditions, and we try to discuss what goes wrong with these cells in specific pathological conditions, such as Alzheimer disease, prion diseases and amyotrophic lateral sclerosis.

Introduction

The functioning of the central nervous system (CNS) has been long explained by a neuron-centric vision of the CNS, attributing all the main information processing activities to neuronal cells. Yet, more recent work has challenged this concept, revealing that the non-neuronal cell component of the nervous system, particularly glial cells, also perform a dynamic range of functions that are essential for the development and physiology of the brain and spinal cord.[1-3] Thus, there is growing consensus that a more integrated level of neuroglial interactions needs to be considered in order to have a comprehensive overview of brain functions and dysfunctions.

The most abundant glial cell type in the mammalian brain is represented by the astrocytes, which constitute up to 50% of its volume.[4] These cells have been traditionally regarded just as passive housekeepers apt to preserve the optimal microenvironment for neuronal function and survival. However, the recent acknowledgment of a broader range of previously unrecognized properties and activities has dramatically changed this view, introducing the idea that astrocytes contribute to the performance of the CNS.[1,5] The best-characterized astroglial cells, in terms of morphology and organization, are protoplasmic astrocytes, located in the parenchyma of the gray matter. Intracellular injection of fluorescent dyes revealed that protoplasmic astrocytes present a peculiar anatomical organization, based on the occupancy of exclusive, non-overlapping territories.[6-8] Within its own territory, each astrocyte extends highly ramified processes that establish numerous connections with the adjacent cellular populations, i.e., neurons, other glial cells and the blood vessels. This intimate spatial relationship with both neurons and the blood vessel cells puts astrocytes in an ideal position to integrate the neural circuitry with the local microcirculation.[9-11] As a consequence, astrocytes emerge as the main effectors of the CNS homeostasis. They supply energy metabolites to neurons,[11] regulate the blood flow and the blood-brain barrier,[12] and control the levels of extracellular ions, neurotransmitters and fluids.[13-15] In addition, the expression of functional receptors on their plasma membrane allows astrocytes to sense neurotransmitters spilled over from nearby synaptic sites. In turn, astrocytes can respond to neurons by Ca^{2+}-dependent release of various gliotransmitters (i.e., transmitters of glial origin, as opposite to neurotransmitters), which can influence neuronal and synaptic functions.[1-3] This close anatomical and functional interactions between astrocytes and neurons prompted the formulation of a new concept of synaptic physiology, i.e., the tripartite synapse, wherein the flow of information involves the processes of perisynaptic astrocytes in addition to presynaptic and postsynaptic nerve terminals.[2,16,17]

The multiplicity and complexity of these activities clearly indicate that the correct performance of the astrocytes is crucial for the physiological functioning of the CNS, and its derangement may affect both neuronal activity and survival.

Astrocyte Signaling in Physiology

Because of their inability to generate action potentials and communicate via electrical signals, astrocytes have been long considered as non-excitable cells. Actually, they display a peculiar form of excitability that is based on variations in the intracellular concentration of Ca^{2+} ions ($[Ca^{2+}]_i$). Originally described in the early nineties in cell cultures,[18] astroglial $[Ca^{2+}]_i$ changes have been more recently characterized in acute brain slices and in vivo. Thus, it has been determined that astrocytic $[Ca^{2+}]_i$ elevations can occur spontaneously[19-23] or can be evoked in response to various physiological and pharmacological stimulations.[24-35]

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Although the physiological significance of such intracellular Ca^{2+} rises has not been fully clarified, it appears that astroglial $[Ca^{2+}]_i$ signaling represents a composite mechanism by which astrocytes control a broad variety of cellular processes, including the exocytotic release of various gliotransmitters. The discharge of such mediators seems to be generally triggered by activation of inositol 1,4,5 triphosphate [Ins(1,4,5)P_3]-generating G-protein-coupled receptors (GPCRs), followed by Ins(1,4,5)P_3-mediated release of Ca^{2+} from the endoplasmic reticulum (ER) stores.[36-40]

Remarkably, such regulated release of gliotransmitters appears to be relevant for the astrocyte-to-neuron communication as well as for the performance of the CNS, as indicated by several studies performed in various experimental paradigms. Thus, the release of the gliotransmitter glutamate from astrocytes was described to exert various forms of neuromodulatory activity in different brain regions.[19,31-33,41-46] Furthermore, the discharge of other glial mediators, particularly D-serine and adenosine, was reported to control the induction of long-term potentiation (LTP) at nearby excitatory synapses[47] as well as to modulate a number of animal behaviors,[48-50] respectively. Among the GPCRs competent to initiate the astrocytic release of gliotransmitters, there are group I metabotropic glutamate receptors (mGluRs), CXCR4 chemokine receptors and $P2Y_1$ purinergic receptors ($P2Y_1$Rs).[38,51-54] Notably, Ca^{2+}-dependent release of glutamate from cultured astrocytes, in response to stimulation of the CXCR4 chemokine receptors or the purinergic $P2Y_1$ receptors, was reported to be controlled by pro-inflammatory mediators, such as prostaglandins and cytokines, particularly the tumor necrosis factor α (TNFα).[32,38,51,52] Since the levels of these mediators are subjected to dramatic increases in several neurodegenerative diseases, it is reasonable to postulate that the molecular pathway controlling the glial release of glutamate can become over stimulated in pathological conditions, and this may perturb the astrocyte-to-neuron signaling and, possibly, trigger neurodamaging events.

Altogether, this scenario suggests that understanding in depth the versatility of both the astrocytes and the glial-neuronal interactions may be critical to gain new insights into the molecular basis of various neurodegenerative processes.

Astrocyte Signaling in Pathology

Several lines of evidence indicate that astrocytes do not react in a stereotyped fashion to all forms of injury and disease, but the mode and the extent of the astrocytic reaction can vary in dependence of both the severity of the insult and the context of the injury site. The most evident reaction of the astrocytes to several neurodegenerative diseases is represented by a vigorous activation, a condition commonly known as "reactive astrocytosis."[55,56]

While the specific factors and cellular interactions that govern the activation of astroglial cells remain mostly elusive, it is clear that the shift from the resting to the activated phenotype is associated with morphological and biochemical changes of the astrocytes.[55,56] Little is known about the impact that these alterations have on the astrocyte signaling. However, evidence indicates that reactive astrocytes are endowed with a plethora of molecules that are undetectable or present at lower levels in quiescent astroglia.[55,56] Among these molecules, there are several cytokines and enzymes involved in the metabolic pathways of arachidonic acid, and thus in the generation of eicosanoids.[55,56] This finding is particularly interesting considering that some of these molecules, such as TNFα and prostaglandins, control the Ca^{2+}-dependent release of glutamate from astrocytes,[32,38,51,52] which has been implicated in the excitotoxic death of neuronal cells in vitro, in neuron-glia co-culture systems.[38] Additional alterations of reactive astrocyte physiology have been described in a pioneer study by Aguado and colleagues. These authors reported that while spontaneous $[Ca^{2+}]_i$ transients are a common feature of resting astrocytes, they are lost in reactive astrocytes in situ.[21] Since $[Ca^{2+}]_i$ oscillations control astrocytic gliotransmitter release and may synchronize neuronal network activity, such defect appears relevant to CNS function, and should be also taken into consideration when studying neurodegenerative mechanisms.

Another aspect that should be considered carefully is that, in specific pathological circumstances, the reaction of astrocytes to injury or disease may take the form of atrophy or degeneration. This phenomenon was interpreted by some as a defensive mechanism to stem the overwhelming reaction of reactive astrocytes.[57] However, different causative hypotheses can be postulated, including the possibility that diseased astrocytes may become susceptible to physiological stimuli, and this progressively leads to the deterioration of their health conditions.[58,59]

On a mechanistic standpoint, the degenerative process of the astrocytes was described to be fine-tuned by a delicate balance between pro-survival and pro-apoptotic factors promoting or preventing astrocyte cell death, respectively.[57,59] In this context, cytosolic Ca^{2+} may gain importance in view of its role in controlling the cell fate under stress conditions. It is generally well established that intracellular Ca^{2+} levels are critical to modulate a variety of cellular responses that are fundamental for a significant number of vital functions. However, cellular Ca^{2+} overload, or perturbation of intracellular Ca^{2+} compartmentalization, can cause cytotoxicity and trigger various forms of cell death.[60-63] Thus, it is reasonable to assume that, in the astrocytes, intracellular Ca^{2+} concentrations may represent a critical switch between life and death. As a consequence, studying astrocytic Ca^{2+} signaling and Ca^{2+}-dependent gliotransmitter release in pathological conditions emerges as a high priority to elucidate the contribution of this glial cell population to neurodegeneration and neurodegenerative diseases.

In this review, we tackle the abnormalities that the astrocytic signaling encounters in specific pathologies of the CNS, such as Alzheimer disease (AD), prion diseases and amyotrophic lateral sclerosis (ALS). While these disorders are often seen as disparate pathologies, evidence indicates that they share prominent common features, including dramatic changes of the astrocytes. According to several observations, these glial alterations are unlikely to play a causal role in the pathogenesis of neurodegenerative disorders. Nevertheless, in transgenic models, glia appear to contribute to disease progression.[64] Thus, clarifying both how the glial responses are triggered under pathological conditions as well as their specific contribution to critical neurodamaging mechanisms may highlight novel cellular and molecular targets for therapeutic intervention.

Alzheimer Disease

Alzheimer disease (AD) is the most common form of progressive dementia in the elderly. Most cases of AD are sporadic, but approximately 1–2% of instances are genetically linked and can be distinguished by the early onset of dementia. The most critical risk factor for the development of AD is age, with the prevalence of cases increasing exponentially after 65 years.[65] On a clinical standpoint, the disease is characterized by the progressive impairment of higher cognitive function, loss of memory and altered behavior, which follow a gradual progression. The major histopathological hallmarks of AD are the deposition of extracellular amyloid plaques and the formation of intracellular neurofibrillary tangles in the brain.[65] These events are accompanied by a progressive neuroinflammatory reaction that involves the activation of glial cells around amyloid plaques.[66,67] In the brain of both AD subjects and animal models, reactive microglia and astrocytes produce high levels of pro-inflammatory cytokines that were proposed to exacerbate neuronal damage.[68-71] As the disease progresses, synapse loss and neuronal cell death become prominent, with the consequent shrinkage of the affected areas, i.e., the entorhinal cortex and the hippocampus. Amyloid plaques mostly consist of aggregated amyloid β (Aβ) peptide generated by the sequential proteolitic cleavages of the amyloid precursor protein (APP) via β- and γ-secretase enzymes.[72] The β-site APP-cleaving enzyme 1 (BACE1) has been identified as the β-secretase that iniziates the production of Aβ peptide.[73] Since neurons express higher levels of BACE1 than astrocytes,[74-76] it was initially proposed that they are the major source of Aβ in brain.[74] However, in both autoptic AD cases and transgenic mice, BACE1 expression and Aβ deposits are not only restricted to neurons, but they co-localize also with astroglial markers.[77-80] This suggests that astrocytes may contribute to the generation of pathological protein aggregates in vivo. In keeping, recent studies in vitro confirmed that, under normal conditions, astrocytes possess the APP-processing machinery for generating Aβ peptide, i.e., APP itself, BACE1 and/or its close homolog BACE2.[76,80,81] Furthermore, Aβ peptide was described to transcriptionally regulate the expression of the astrocytic BACE1 via the calcineurin/nuclear factor of activated T cells 4 (NFAT4) or nuclear factor-κB (NF-κB) signaling pathways,[82,83] suggesting a feed-forward mechanism to maintain or amplify Aβ deposits in astrocytes. More controversial is the issue of reactive astroglia as some studies indicated that cultured astrocytes, activated with cytokine combinations, positively regulate β-secretase activity and Aβ secretion,[84] whereas others found opposite results.[76]

A correlation between the deposition of Aβ protein in the extracellular space of forebrain regions and the progressive decline in cognitive functions was originally established in morphological and biochemical studies in AD patients.[85-87] Subsequent results obtained in various transgenic AD models provided additional evidence that cognitive deficits are the consequence of the synaptic failure induced by Aβ.[88] Synaptic transmission and plasticity is strongly based on Ca^{2+} signals, and these latter appear to be disrupted in AD neurons.[89-91]

Altogether, these observations can be explained by the "calcium hypothesis" of AD according to which the amyloidogenic APP processing may perturb the neuronal Ca^{2+} signaling pathways that are responsible for cognitive functions. Eventually, this can cause the learning and memory deficits that characterize the early stages of AD.[92] Alterations of neuronal Ca^{2+} levels may also influence the metabolism and production of Aβ, a peptide whose accumulation in the brain may further exacerbate Ca^{2+} dyshomeostasis and neurodegeneration.[93]

Besides neurons, important alterations of Ca^{2+} signaling were also described in the astrocytes by two-photon Ca^{2+} imaging in vivo in the cerebral cortex of a mouse model of AD.[94]

While the specific implications of astrocytic Ca^{2+} signaling alterations are not yet known, the existence of such phenomenon suggests that, in AD, the circuital dysfunction may be generalized, involving not only synaptic transmission but also neuron-glia communication. Additional evidence in favor of this idea was provided in vitro, in cell culture experiments.

Administration of Aβ peptides to mixed cultures of hippocampal neurons and astrocytes was in fact described to cause abnormal $[Ca^{2+}]_i$ transients and mitochondrial depolarization in astrocytes, long before any impairment was visible in neurons. Blocking the astrocytic Ca^{2+} protected neurons from delayed cell death,[95,96] thereby establishing a direct correlation between Aβ-dependent alterations of the astrocytic Ca^{2+} signals and neuronal loss in vitro. Furthermore, astrocytic Ca^{2+} variations appear to be critical also for the release of neurotoxic concentrations of the gliotransmitter glutamate initiated by high levels of TNFα.[38] Because this cytokine is highly represented in the CNS of AD patients and transgenic mice,[67,68,70,71] it is plausible to hypothesize that such process may be relevant also for AD-linked neurodamaging events. Our group tested this hypothesis using the PDAPP mice, a transgenic model of AD.[97] In particular, we utilized aged animals (12 months old), presenting abundant amyloid plaque deposition and reactive gliosis in the forebrain, and pre-symptomatic animals (4 months old), with little or no amyloid deposits and no apparent glial alteration. Ca^{2+}-dependent glutamate release from astrocytes was stimulated in brain slices from PDAPP animals and controls by direct application of high concentrations of exogenous TNFα. The amount of glutamate secreted by hippocampal slices from aged PDAPP animals was significantly lower compared with pre-symptomatic mice and age-matched controls.[97] The defect was region-selective as the glutamate release response from cerebellar slices of aged PDAPP mice was identical to that of controls.[97] Altogether, these observations were quite unexpected, particularly in view of previous data in cultures where, in an acute experiment, production of high TNFα levels by reactive microglia strongly potentiated the astrocytic release of glutamate.[38] However, in AD, glial inflammation is a chronic phenomenon, particularly around protein aggregates, where endogenous TNFα levels are presumably constantly high and may over-stimulate receptors, thus causing functional uncoupling.[68,70,71]

At present, the functional significance of this defect is not established. However, one can speculate that a reduced astrocytic glutamate input to neurons may result in weakened connectivity

of excitatory hippocampal synapses.[31,32] Remarkably, TNFα was also reported to critically control glutamatergic gliotransmission in the hippocampus,[32] and astrocytic TNFα-dependent signaling was shown to favor synaptic strength[98] and induce homeostatic synaptic up-scaling[98-100] by promoting insertion of post-synaptic AMPA receptor subunits. Therefore, disruption of TNFα signal-transduction in astrocytes could lead to several alterations converging in a progressive reduction of synaptic efficacy and, possibly, underlie behavioral deficits in AD.

Prion Diseases

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of invariably fatal neurodegenerative disorders affecting humans and a wide range of mammals. An important feature of these disorders is the accumulation in the CNS of PrP^{Sc}, a protease-resistant conformer of the host-encoded cellular prion protein (PrP^{C}), believed to be the main or only constituent of the transmissible agent ("prion").[101-103] Despite considerable attention resulting from its involvement in these disorders, the physiological function of PrP^{C} remains elusive.

Thus, an unsolved issue is whether disease progression is mainly affected by the accumulation of PrP^{Sc} in brain and/or by the loss of PrP^{C} function.[104-106]

Although prion diseases present some common neuropathological traits with AD, the Ca^{2+} pathophysiology has been much less characterized in the context of these disorders, and knowledge appears to be limited to neuronal cells. Thus, receptor-mediated $Ins(1,4,5)P_3$ and $[Ca^{2+}]_i$ responses resulted markedly reduced in scrapie-infected mouse neuroblastoma cells.[107,108]

Furthermore, the ER Ca^{2+} content was shown to be decreased in cells chronically infected with scrapie prions, and this correlated with a higher sensitivity to ER stress-induced cell death.[109] Since a similar reduction in the ER Ca^{2+} levels were recently reported in primary neurons lacking the cellular prion protein, it was postulated that PrP^{C} may be part of the machinery deputed to maintain the intracellular Ca^{2+} homeostasis. As a consequence, the loss of its function, during disease progression, may contribute to Ca^{2+} derangement and synaptic failure.[110] Even though astrocytes were described to be the earliest site of PrP^{Sc} accumulation in brain,[111] no information is currently available on the Ca^{2+} signaling of scrapie-infected astroglia.

The presence of chronically activated glial cells around PrP^{Sc} depositions is a well documented feature in prion diseases.[112-114] While it is not clear whether they play a causative role in the disease, recent data from animal models suggest that activated glia might at least contribute to accelerate its progression. Since high levels of pro-inflammatory cytokines, produced by reactive microglia and astrocytes, are detected in the brain of patients and animal models for these disorders,[115-117] we investigated the TNFα- and Ca^{2+}-dependent release of glutamate from astrocytes on scrapie-infected tissues. Wild-type C57Bl/6J mice were intracerebrally (i.c.) inoculated with the mouse-adapted scrapie strain Rocky Mountain Laboratory (RML). Brains from RML-infected and saline-treated (control) mice were taken at 14, 90 and 135 days post-inoculation (d.p.i.) and used to prepare

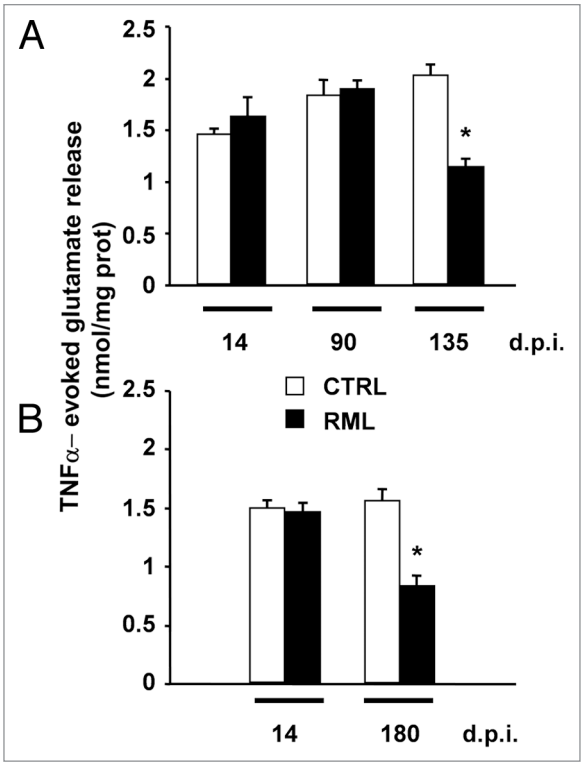


Figure 1. Impaired TNFα-dependent glutamate release from hippocampal astrocytes of scrapie-infected mice. Histograms indicate the glutamate release induced by TNFα (30 ng/ml) in acute hippocampal slices from C57Bl/6J mice intracerebrally 30 μl, (**A**) or intraperitoneally 100 μl, (**B**) inoculated with 1% homogenate from saline-treated (CTRL) or RML-infected, terminally ill CD1 mouse brains. Brains were taken at 14, 90 and 135 days post intracerebral inoculation (d.p.i.) and at 14 and 180 days post intraperitoneal inoculation (days to terminal disease: i.c.: 164 ± 3; i.p.: 209 ± 5). Acute hippocampal slices were prepared and the amount of glutamate released upon stimulation with TNFα was measured using a previously described enzymatic assay.[97] Data are expressed as mean ± s.e.m. (n = 3–6 animals for each experimental group). *p < 0.05 vs. CTRL, two-way ANOVA followed by Scheffe's F-test.

acute slices. Stimulation of scrapie-infected hippocampal slices with exogenous TNFα resulted in a significant reduction of the release of glutamate from astrocytes at the early symptomatic stage of 135 d.p.i. when compared with the pre-symptomatic phases and to slices from age-matched control animals (**Fig. 1A**). Impairment in the astrocytic release of glutamate correlated with a prominent astrocytosis (**Fig. 2**) and the accumulation of both the proteinase K-resistant PrP^{Sc} and infectivity in the hippocampus (**Fig. 3**). Interestingly, such result could not be ascribed to a local neuroinflammatory reaction triggered by intracerebral manipulation and injection of scrapie prions, because we could confirm similar results in hippocampal slices from mice intraperitoneally (i.p.) challenged with RML at the early symptomatic stage of 180 d.p.i. when compared with slices from younger or control animals (**Fig. 1B**) (unpublished observations).

Because these results are fully consistent with the data obtained on the PDAPP model of Alzheimer disease, one may infer that the alterations of the TNFα- and Ca^{2+}-dependent glutamate release from astrocytes cannot be a distinctive feature of

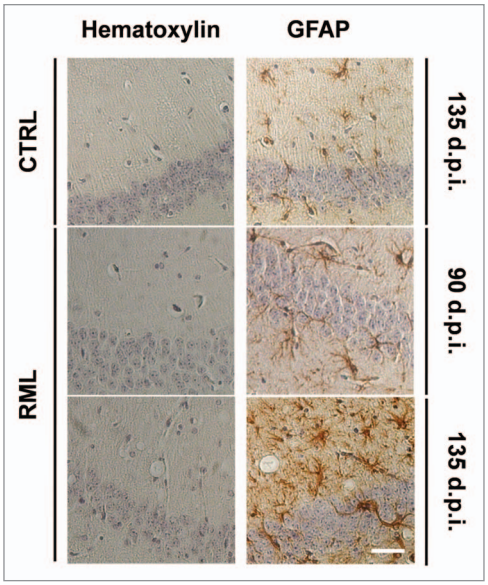
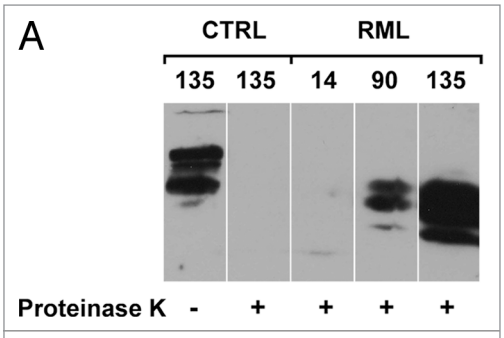


Figure 2. Progressive astrocytosis and spongiosis in the hippocampus of scrapie-infected mice. Coronal sections from saline-treated (CTRL) and RML-infected animals (i.c.) at 90 and 135 d.p.i were stained with hematoxylin alone or hematoxylin with the astrocytic marker glial fibrillary acidic protein (GFAP). Stainings highlight spongiosis and pronounced astrocytosis in the hippocampus of RML-infected mice at 135 d.p.i. Images are representative of three animals. Scale bar, 50μm.



B

D.p.i.	Days to death	n/nt
14	> 428	0/5
90	77 ± 2	5/5
135	71 ± 1	6/6

Figure 3. PrP^{Sc} accumulation and prion titers in the hippocampus of RML-infected mice. (**A**) Hippocampal homogenates from saline-treated (CTRL) or RML-infected mice (i.c.) at 14, 90 and 135 d.p.i. were treated in the presence (+) or in the absence (-) of proteinase K to distinguish native PrP^{C} from the proteinase K-resistant PrP^{Sc} and analyzed by western blotting (n = 3). (**B**) Prion titers in the hippocampus of infected animals at 14, 90 and 135 d.p.i. were determined by inoculating homogenates i.c. into CD-1 indicator mice. Data highlight a progressive increase in the amount of PrP^{Sc} and infectivity in the hippocampi of RML-infected mice at 135 d.p.i. n/nt = number of mice with scrapie/ total number of mice inoculated.

a specific pathology, but it is rather a peculiarity of chronically activated astrocytes.

Amyotrophic Lateral Sclerosis

Another disorder of the nervous system that results particularly interesting because of the overt involvement of glial cells in the development and progression of the disease is amyotrophic lateral sclerosis (ALS), a pathological condition characterized by the progressive loss of corticospinal and spinal motor neurons. Although multiple genes and genetic loci have been recently linked to this disorder,[118-120] most of the current knowledge on ALS pathogenesis is based on the discovery of mutations in the enzyme Cu-Zn superoxide dismutase (SOD1)[121] and the subsequent generation of transgenic animal models.[122-127]

While the primary toxic property of mutant SOD1s remains unresolved, a hint of the cascade of events implicated in motor neuron degeneration came by the landmark observation that death of the motor cells is a non-cell-autonomous process, but instead involves interaction with neighboring non-neuronal cells, particularly microglia and astrocytes.[128] Massive activation of microglia and astrocytes in areas of motor neuron loss was reported in both sporadic and familial human cases, as well as in transgenic animal models.[129-131] Though microglial alterations were shown to be directly implicated in favoring disease progression in vivo,[130,132] silencing mutant SOD1 expression in astrocytes was reported to affect disease onset and progression in transgenic mice.[131,133] Furthermore, transplantation of mutant SOD1-expressing glial progenitors, capable of differentiating into astrocytes, into the spinal cord of wild-type rats induced motor neuron degeneration and disease symptoms in vivo, definitively confirming a causative role for these cells in ALS.[134]

A peculiar histopathological abnormality in tissues of ALS patients is the presence of ubiquitin inclusions within astrocytes.[135] Noteworthy, similar features are shared by mutant SOD1 mice, which show protein aggregates made of SOD1, ubiquitin and/or activated caspase-3 in astroglial cells.[124,136] To clarify the impact of such inclusions on the astrocyte performance in ALS, our group performed a thorough histopathological analysis of the lumbar tract of the spinal cord from transgenic mice carrying the Gly93→Ala substitution in the human SOD1 amino acid sequence ($hSOD1^{G93A}$). We localized a subpopulation of astrocytes harboring protein inclusions specifically in the neighborhood of motor cells. These astrocytes displayed morphological and biochemical features reminiscent of degenerating cells,[58] including a spheroid cell body with increased diameter and a reduced number or even absence of GFAP-positive cell processes.

When present, such processes appeared short and abnormally thick compared with those of the normal astrocytes. Degenerating astroglial cells were first observed at the pre-symptomatic stage, when motor neurons show axonal damage but are still alive.[137] Their number significantly increased concomitant with the onset of neuronal degeneration and the appearance of ALS symptoms. We then studied the mechanism underlying astrocyte degeneration in cultured spinal cord astrocytes and surprisingly found that mutant SOD1s, either G93A or G85R, do not exert a direct

pro-apoptotic action, but rather make the astrocytes vulnerable to glutamate, even at physiological concentrations. The glutamatergic mechanism responsible for the deleterious effect was found to involve metabotropic glutamate receptor 5 (mGluR5) signaling. Thus, blockage of this receptor reduced apoptosis of mutant SOD1-expressing astrocytes in response to glutamate challenges. Moreover, administration of a mGluR5 antagonist in vivo reduced astrocyte degeneration in the lumbar spinal cord, delayed the appearance of ALS symptoms and extended survival in hSOD1^{G93A} transgenic mice.[58]

In normal astrocytes, the activation of mGluR5 triggers the formation of Ins(1,4,5)P_3 and the consequent release of Ca^{2+} from the ER, resulting in intracellular Ca^{2+} oscillations.[138,139] Thus, we investigated the astrocytic Ca^{2+} response downstream mGluR5 in mutant SOD1-expressing astrocytes. We found that the mutant cells responded to the receptor stimulation with an aberrant and persistent Ca^{2+} release from the intracellular stores that correlated with cytochrome *c* release from mitochondria and astrocyte degeneration.[59] These results are fully consistent with the description of mitochondrial dysfunction in mutant SOD1-expressing astrocytes by other authors.[140] Since the Bcl-2 family members were reported to exert their anti-apoptotic activity by fine-tuning intracellular Ca^{2+} signaling through direct interaction with the Ins(1,4,5)P_3 receptors [Ins(1,4,5)P_3Rs], we then investigated the impact of the BH4 domain of Bcl-X_L on astrocyte Ca^{2+} signaling by exploiting a biologically active BH4 peptide fused to the HIV-1 TAT protein (TAT-BH4). We realized that TAT-BH4 modulates the Ins(1,4,5)P_3R-dependent Ca^{2+} release from the ER, and restores spontaneous Ca^{2+} oscillations in mutant SOD1-expressing astrocytes. This tight control of Ins(1,4,5)P_3Rs by the peptide prevents the mGluR5-dependent aberrant release of Ca^{2+} from the intracellular stores, precludes the release of cytochrome *c* from mitochondria and protects the cells from excitotoxic damage. Furthermore, chronic administration of TAT-BH4 in vivo, to hSOD1^{G93A} transgenic mice, reduces degeneration of spinal cord astrocytes and shows a positive impact on disease manifestations.[59]

But what is the relevance of these findings in the context of ALS? And what are their repercussions on motor neurons? As mentioned above, astrocytes are intimately associated with synapses and can sense neurotransmitter released during synaptic activity.[1] Therefore, we infer that spinal cord astrocytes, endangered by the expression of ALS-linked mutant SOD1s, become vulnerable to physiological glutamate concentrations released at neighboring synapses and start to degenerate. This in turn may deprive the neighboring motor neurons of the optimal microenvironment and accelerate their degeneration in an interactive process of reciprocal damage.

Conclusions

The deposition of misfolded protein aggregates in regions of neuronal degeneration represents a typical feature of most brain disorders. Some of the findings briefly summarized in this review, indicate that, such protein aggregates not only accumulate in neurons, but they are present also within astroglial cells, possibly suggesting that astrocytes themselves may be functionally compromised in pathological conditions. In keeping with this hypothesis, some astroglial pathways, such as those controlling the astrocytic Ca^{2+} signaling and the Ca^{2+}-dependent release of gliotransmitters, appear to be disrupted in various CNS disorders. Considering that these processes are critically involved in astrocyte survival as well as in the modulation of synaptic activity, it becomes clear that glial derangements in neurodegenerative conditions should not be considered only as epiphenomena or late reactions to neuronal injury, but rather as intrinsic components of the pathological process. Additional steps are certainly necessary to improve our understanding of the astrocyte pathophysiology. Nevertheless, the awareness that astrocytes are actively involved in the CNS functioning offers a wide range of new possibilities to unravel physiological and pathological mechanisms. This may open new perspectives for original therapeutic strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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