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Review

The active role of astrocytes in synaptic transmission

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Abstract. In the central nervous system, astrocytes form an intimately connected network with neurons, and their processes closely enwrap synapses. The critical role of these cells in metabolic and trophic support to neurons, ion buffering and clearance of neurotransmitters is well established. However, recent accumulating evidence suggests that astrocytes are active partners of neurons in additional and more complex functions. In particular, astrocytes express a repertoire of neurotransmitter receptors mirroring that of neighbouring synapses. Such receptors are stimulated during synaptic activity and start calcium signalling into the astrocyte network. Intracellular oscillations and intercellular calcium waves represent the astrocyte's own form of excitability, as they trigger release of transmitter (i.e. glutamate) via a novel process sensitive to blockers of exocytosis and involving cyclooxygenase eicosanoids. Astrocyte-released glutamate activates receptors on the surrounding neurons and modifies their electrical and intracellular calcium ($[Ca^{2+}]_i$) state. These exciting new findings reveal an active participation of astrocytes in synaptic transmission and the involvement of neuron-astrocyte circuits in the processing of information in the brain.

Key words. Astrocytes; neuron-glia interaction; synaptic transmission; glutamate; calcium signalling; prostaglandins; transmitter release.

Introduction

The 100 billion neurons composing the mammalian central nervous system (CNS) are surrounded by a 10-fold higher number of glial cells. These cells were first identified in the middle of the 19th century by the German anatomist Rudolf Virchow, who described them as 'a real cement that binds nervous elements together'. Thus, he named this category of cells neuroglia (literally 'nerve glue').

Subsequent studies showed that glial cells in the CNS can be divided in several subclasses, such as astrocytes, oligodendrocytes and microglia, each of them playing distinct roles in brain function [1]. Nevertheless, due to their supposed subsidiary role with respect to neurons, for decades glial cells were very little studied. Only in the last 20 years has an increasing amount of evidence outlined that glial cells, despite generating neither nor conducting action potentials nor apparently forming synapses with neuronal cells, interact with neurons in a more complex manner than simply providing structural, metabolic and trophic support. These findings have encouraged more detailed research on glial cells, and very recently important breakthroughs have been made on the role played by astrocytes in brain function, revealing that these cells are dynamic and capable of rapid reciprocal signalling with neurons during synaptic activity. The present review recapitulates these exciting new findings, emphasizing in particular the emerging views on astrocyte-neuron communication at glutamatergic synapses (for other recent reviews see [2–11]).

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Glutamate released during synaptic activity triggers $[Ca^{2+}]_i$ elevation in astrocytes

Excitatory synapses are often ensheathed by astrocyte processes which express clusters of transporters for the transmitter glutamate. Indeed, several recent results support the view that astrocytes play the primary role in the clearance of synaptically released glutamate and in the maintenance of its extracellular concentration at a physiological low level. In addition, other critical neuron-astrocyte interactions are initiated by uptake of synaptic glutamate into the astrocytes (fig. 1). However, for years neuroscientists thought that astrocytes were unresponsive to excitatory neurotransmitters and only neurons could respond with depolarization and excitation. In the middle 1980s Bowman and Kimelberg [12] reported that L-glutamate depolarizes cultured astrocytes, introducing the idea that 'electrophysiological effects of excitatory amino acids may not be exclusively a neuronal property'. In the following years several groups found that astrocytes express a variety of neurotransmitter receptors, including ionotropic (s)-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate and metabotropic glutamate receptors (mGluRs; for a review see [13]).

What kind of intracellular events could the activation of these receptors generate in astrocytes? The first important results were reported by S. Smith and co-workers [14], who demonstrated that application of exogenous glutamate to cultured hippocampal astrocytes elevated internal calcium and triggered $[Ca^{2+}]_i$ oscillations lasting several minutes. The use of agonists selective for different GluR types allowed dissecting out of distinct components of the response. Thus, in the absence of external Ca^{2+}, kainate (which acts on the AMPA/

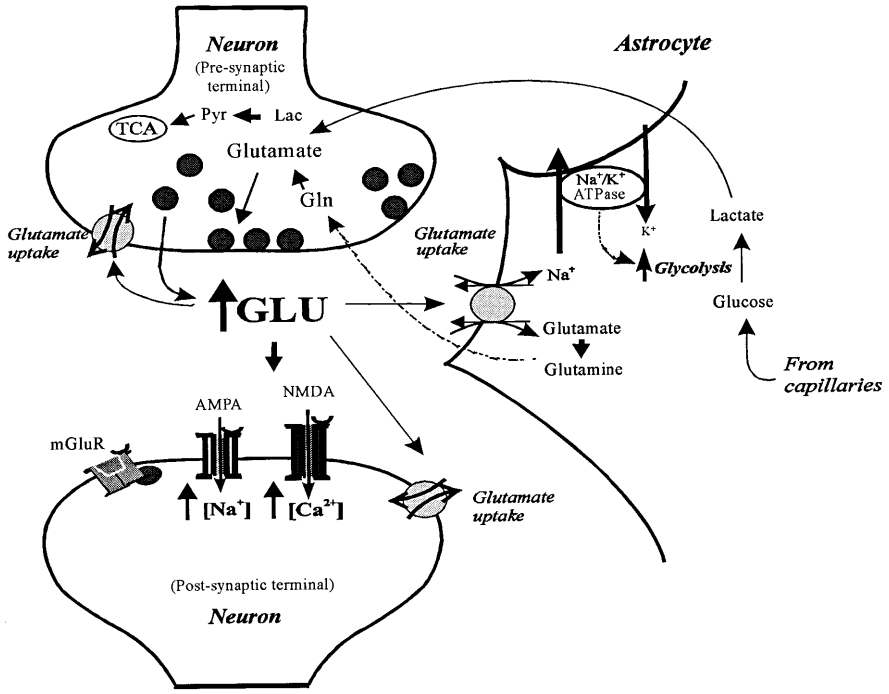

Figure 1. The role of glutamate transport in neuron-astrocyte coupling. Glutamate released during synaptic activity is taken up by specific transporter proteins located on neuronal plasma membranes but also on astrocytes (for reviews see [a, b]). Once taken up by the astrocytes, glutamate can be metabolized to glutamine by glutamine synthetase, an enzyme present only in these cells, and, in this inactive form, is shuttled back to neurons to replenish the synaptic pool. In addition, glutamate uptake into the astrocytes starts a reverse process of energy supply from astrocytes to neurons (for review see [c]). In particular, cotransport of Na^+ with glutamate into the cell during the uptake cycle activates the Na^+/K^+ ATPase pump, with consequent hydrolysis of ATP, increased glycolysis and uptake of glucose from blood vessels. During the glycolytic process, lactate is produced and then delivered to neurons by specific unidirectional transport, and enters the tricarboxylic acid (TCA) cycle. It has been proposed that uptake of glutamate and release of lactate are coupled, with a 1:1 stoichiometry, which would tightly link the level of synaptic activity to the astrocytic metabolic supply [d]. For clarity, the present scheme reports only postsynaptic GluRs. However, GluR subtypes are present also presynaptically and on astrocytes (see text and fig. 2). [a] Eliasof S., Arriza J. L., Leighton B. H., Kavanaugh M. P. and Amara S. G. (1998) Excitatory amino acid transporters of the salamander retina: identification, localization and function. J. Neurosci. **18:** 698–712. [b] Trotti D., Danbolt N. C. and Volterra A. (1998) Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration? Trends Pharmacol. Sci. **19:** 328–334. [c] Tsacopoulos M. and Magistretti M. J. (1996) Metabolic coupling between glia and neurons. J. Neurosci. **16:** 877–885. [d] Magistretti S. J., Pellerin L., Rothman D. L. and Shulman R. G. (1999) Energy on demand. Science **283:** 496–497.

kainate receptors selectively) was inactive, whereas quisqualate (which acts both on AMPA/kainate and mGluRs) and glutamate triggered short-lasting $[Ca^{2+}]_i$ oscillations. The authors concluded that glutamate increased $[Ca^{2+}]_i$ in astrocytes by acting on two receptor types, one mobilizing calcium from intracellular stores (the 'quisqualate-preferring'), the other promoting calcium influx through the cytoplasmic membrane (the 'kainate-preferring'). They also showed that Ca^{2+} signals spread through a network of contacting astrocytes in the form of 'calcium waves' and proposed that intercellular propagation of calcium signals could depend on a second messenger diffusing from one cell to another via the gap junction channels (see below).

Smith and co-workers continued their studies on organotypic hippocampal slices so as to investigate interactions between neurons and astrocytes forming their natural connections [15]. Electrical stimulation of the mossy fiber pathway induced calcium signals in the surrounding astrocytes with features utterly similar to those observed in culture. However, although glutamate is the primary neurotransmitter released from these hippocampal afferents, the researchers did not exclude that astrocyte responses could be induced by collateral events to the exocytotic secretion of glutamate (e.g. potassium efflux from the depolarised fibers or co-released factors such as adenosine triphosphate (ATP)).

A more conclusive indication that astrocytes respond directly to synaptically released neurotransmitter (i.e. glutamate) was provided few years later [16]. By using acute hippocampal slices, Porter and McCarthy showed that electrical stimulation of CA3 Schaffer collaterals resulted in $[Ca^{2+}]_i$ elevation in the nearby astrocytes of the stratum radiatum. They suggested that the phenomenon was indeed induced by the synaptically released glutamate, since it was antagonized by both tetrodotoxin, which blocks the evoked release, and by a selective mGluR antagonist. Interestingly, these authors also showed that more intense neuronal stimulations evoked larger astrocytic calcium responses, that could now be prevented only by blocking both metabotropic and ionotropic GluRs at the same time. This observation suggested that activation of astrocytes by synaptically released neurotransmitters could be finely modulated, depending on the intensity and frequency of neuronal activity. The synaptically released glutamate spills over from synapses and acts on nearby astrocytes. Repetitive firing of neurons is known to trigger more synaptic release of glutamate than single spikes because of paired-pulse facilitation. Thus, repetitive stimuli may trigger more intense astrocytic responses. The capacity of astrocytes to respond to synaptic transmitters in an activity-dependent manner was confirmed by G. Carmignoto and co-workers [17], who also showed plasticity of the astrocyte response.

In addition to glutamate, other transmitters released during synaptic activity (e.g. norepinephrine [18] and GABA [19]) were shown to evoke $[Ca^{2+}]_i$ rises in astrocytes of hippocampal slices, thus indicating that neuron-to-astrocyte signalling during synaptic transmission is not restricted to glutamatergic synapses.

$[Ca^{2+}]_i$ elevation in astrocytes starts signalling to neurons

What is the functional meaning of the calcium signalling activated by synaptically released transmitters in astrocytes? Does it serve to inform the astrocyte network of the surrounding synaptic activity or does it also start an astrocyte response to it?

Two papers published in 1990 indicated that astrocytes are capable of releasing transmitters such as glutamate, although in pathological rather than physiological conditions. Starting from the observation that in case of ischemia or anoxia the physiological ion gradients across the plasma membrane are overturned, the group of D. Attwell [20] demonstrated that inversion of the ion forces driving glutamate uptake leads to a nonvesicular release of glutamate from cultured Muller cells. In addition, Kimelberg and co-workers [21] showed that swelling of astrocytes resulting from exposure to a hypotonic buffer induces a Ca^{2+}-independent osmotic release of amino acids such as glutamate, aspartate and taurine. However, the idea that astrocytes could physiologically signal to neurons via a regulated Ca^{2+}-dependent glutamate release was far from coming, and still in 1993 it was commonly believed that 'glial cells synthesize and release a variety of molecules functioning as neurotransmitters but, in contrast to neurons, release is not Ca^{2+}-dependent' [22].

Only 1 year later, a milestone paper by the group of P. Haydon [23] announced that $[Ca^{2+}]_i$ elevation in astrocytes results in glutamate release capable of activating the surrounding neurons of hippocampal cultures. The authors found that stimulation of astrocytes with bradykinin triggered $[Ca^{2+}]_i$ elevation and glutamate release. Bradykinin elevated $[Ca^{2+}]_i$ also in neurons co-cultured with the astrocytes but not in solitary ones. $[Ca^{2+}]_i$ elevations in neurons followed those in astrocytes and were mediated by glutamate released from the astrocytes, being abolished in the presence of GluR antagonists. This was the first demonstration that astrocytes signal to neurons in response to a physiological stimulus (i.e. activation of a ligand receptor) by means of a Ca^{2+}-dependent glutamate release process.

In the same year, another important paper by M. Nedergaard [24] provided complementary evidence that astrocytes can signal to neurons via a calcium-dependent process. The author induced Ca^{2+} waves in confluent

cultured astrocytes by means of an electrical impulse and showed that when the wave front reached an overlying neuron, the $[Ca^{2+}]_i$ in the neuron was rapidly elevated. Unlike P. Haydon and co-workers [23], Nedergaard suggested that direct gap-junction coupling rather than chemical transmitter release mediated the astrocyte-neuron signalling since octanol, actually a quite unspecific inhibitor of gap-junctions, prevented it.

Thus, on the one hand, astrocytes respond to synaptically released glutamate with $[Ca^{2+}]_i$ elevations; on the other, $[Ca^{2+}]_i$ elevations in astrocytes can lead to activation of surrounding neurons, whatever the mediator. Can the circle be closed and the existence of a coordinated bidirectional neuron-astrocyte signalling be disclosed? Recent work in our lab [25] as well as in other labs provides some of the first supporting evidence.

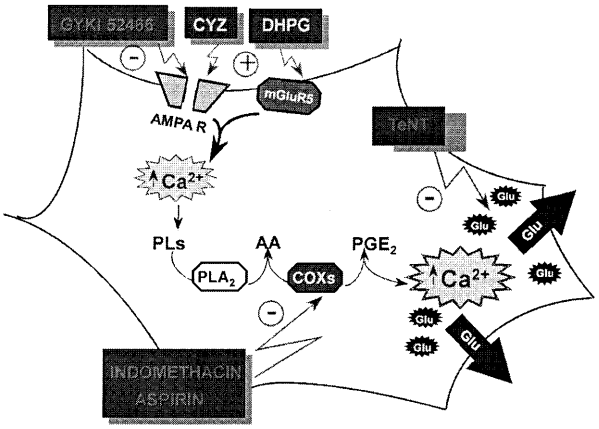

Figure 2. Signal-transduction pathways of the Ca^{2+}-dependent glutamate release process in astrocytes in response to glutamatergic activation. Coactivation of AMPA/kainate and mGlu receptors induces Ca^{2+}-dependent activation of phospholipase A_2 (PLA_2) and mobilization of arachidonic acid (AA) from membrane phospholipids. Cyclooxygenase enzymes (COXs) then metabolize AA to prostaglandins, in particular to PGE_2, that further elevates $[Ca^{2+}]_i$ and induces glutamate release via a tetanus neurotoxin (TeNT)-sensitive mechanism, possibly from vesicular stores. In addition, PGE_2 could act paracrinally on neighbouring astrocytes, thereby amplifying the glutamate release response. Pharmacological studies implicate AMPA-preferring receptors of the AMPA/kainate family in the activation of the mechanism because of inhibition of the release by GYKI 52466 and stimulation by cyclothiazide (CYZ). Involvement of mGluR5 is suggested by (i) the releasing efficacy of the selective group I mGluR agonist (*R*, *S*)-3,5-dihydroxyphenilglycine (DHPG), and (ii) the preferential expression of this receptor subtype in astrocytes with respect to the other group I receptor, mGluR1. The critical role of PGs is demonstrated by inhibition of glutamate release in the presence of the COX blockers indomethacin and aspirin.

Glutamatergic activation of astrocytes triggers Ca^{2+}-dependent glutamate release via a novel signal-transduction pathway and consequent $[Ca^{2+}]_i$ elevations in neurons

At the end of 1997, G. Carmignoto and co-workers [17] obtained preliminary data in support of a two-way glutamatergic signalling system between astrocytes and neurons in situ. These authors reported that stimulation of neuronal afferents in acute cortical and hippocampal slices induced $[Ca^{2+}]_i$ oscillations in astrocytes, most likely originating from glutamate released from the presynaptic terminals and acting on astrocyte mGluRs. mGluR-dependent $[Ca^{2+}]_i$ oscillations in astrocytes were followed by $[Ca^{2+}]_i$ increases in surrounding neurons which were variably sensitive to ionotropic GluR antagonists, suggesting that the neuronal responses could be at least in part indirect, triggered by glutamate released from the astrocytes.

In parallel, our group had addressed the question whether $[Ca^{2+}]_i$ elevation in response to synaptically released glutamate and Ca^{2+}-dependent liberation of glutamate in astrocytes are linked by a cause-effect relation [25]. Using a specific enzymatic assay as detector of endogenous glutamate release, we tested glutamatergic stimulations of cultured cortical astrocytes. Activation of mGluRs with (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (t-ACPD) indeed resulted in glutamate release from the astrocytes, whereas activation of AMPA/kainate receptors with AMPA had no effect. However, if AMPARs were activated coincidentally to mGluRs, an extremely potent and rapid release of glutamate was observed. The specific GluR subtypes involved in the response were further characterized pharmacologically. With regard to mGluRs, the use of group I, II and III specific agonists demonstrated a selective involvement of group I mGluRs. In particular, mGluR5 rather than mGluR1 is expressed by astrocytes both in vitro and in situ [13] and has been reported to elicit $[Ca^{2+}]_i$ oscillations in these cells [26]. As for AMPA/kainate receptors, they can be divided in AMPA-preferring and kainate-preferring, depending on the specific subunit composition [13]. AMPA-preferring subunits, rather than kainate ones, are involved in glutamate release from astrocytes, as release is sensitive to modulation by cyclothiazide (potentiation) and GYKI 52466 (inhibition), two agents acting specifically on AMPARs (fig. 2).

The next critical question concerned the mechanism by which activation of GluRs triggers glutamate release from the astrocytes. As mentioned earlier, astrocytes may release glutamate via either Ca^{2+}-dependent [23] or Ca^{2+}-independent processes [20, 21]. We excluded Ca^{2+}-independent mechanisms such as reversal of glutamate uptake or osmotic release by specific experiments. Accordingly, we found that GluR-dependent glutamate release is strictly Ca^{2+}-dependent, being drastically reduced either in the presence of 1,2-bis(2-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid te-

trakis (acetoxymethyl) ester (BAPTA/AM), which buffers $[Ca^{2+}]_i$, or in the absence of external Ca^{2+}. Interestingly, such release was occlusive with the Ca^{2+}-dependent mechanism activated by bradykinin receptors, whereas it was totally additive to the Ca^{2+}-independent mechanism mediated by glutamate transporters, suggesting two important considerations: (i) distinct neuroligand receptors on astrocytes may be coupled to a common Ca^{2+}-dependent transduction pathway leading to glutamate release; (ii) Ca^{2+}-dependent and Ca^{2+}-independent release mechanisms might utilize distinct and independent glutamate pools.

We then focused on the Ca^{2+}-dependent signal-transduction pathway linking GluR activation to glutamate release. Work by the group of J. Bockaert in the early 1990s had shown that joint (but not independent) stimulation of mGluRs and AMPARs in striatal neurons induced Ca^{2+}-dependent activation of phospholipase A_2 (PLA_2) and arachidonic acid release [27]. The contribution by AMPARs was found to be related to the induction of a highly localized Na^+ influx leading to reversal of Na^+/Ca^{2+} exchange, which in turn caused potent activation of PLA_2, in the presence of simultaneous $[Ca^{2+}]_i$ elevation by mGluRs [28]. Since it was reported that not only neurons but also astrocytes respond to glutamate with release of arachidonic acid [29], we wondered whether this pathway plays a role in the GluR-dependent glutamate release process. Indeed we found that (i) coactivation of mGluRs and AMPARs rapidly triggers Ca^{2+}-dependent release of ^{3}H-arachidonate from astrocyte membranes (a weaker effect is also seen upon stimulation of mGluRs, but not of AMPARs), and (ii) specific PLA_2 inhibitors block glutamate release induced by costimulation of AMPARs and mGluRs. In principle, PLA_2 activation in astrocytes could generate many bioactive lipid mediators, including cyclooxygenase, lipoxygenases and epoxygenase eicosanoids as well as platelet aggregating factor (PAF). To evaluate the involvement of specific products, we tried a pharmacological dissection between the several enzymatic pathways using specific inhibitors. The results of these experiments were clear since cyclooxygenase blockers (i.e. indomethacin and aspirin) potently inhibited glutamate release stimulated by coadministration of AMPA and t-ACPD, whereas lipoxygenases and epoxygenase inhibitors and a PAF receptor antagonist were totally ineffective. Accordingly, the three major cyclooxygenase metabolites of astrocytes, prostaglandins (PG) D_2, E_2 and $F_{2\alpha}$, all induced rapid glutamate release, with PGE_2 being the most effective; in addition, we observed formation of PGE_2 immediately after coactivation of AMPARs and mGluRs. Thus, PGE_2 is the candidate mediator of the Ca^{2+}-dependent glutamate release process of astrocytes. Surprisingly, we found that application of PGE_2 to astrocytes produces potent $[Ca^{2+}]_i$ elevation and that this $[Ca^{2+}]_i$ elevation is critical for glutamate release. Overall, these experiments reveal that astrocytes possess a completely novel pathway for Ca^{2+}-dependent transmitter release based on a complex and apparently bimodal dynamics of calcium signalling where eicosanoids play the central role: following costimulation of AMPARs and mGluRs, $[Ca^{2+}]_i$ elevation recruites PLA_2 activity (e.g. by translocation of the cytosolic enzyme to the internal membranes), leading to arachidonate release and production of PGs which further elevate $[Ca^{2+}]_i$ and boost the release of glutamate (fig. 2). PGs could increase $[Ca^{2+}]_i$ in the cell where they are generated and/or on neighbouring ones, acting as poracrine amplificatory factors. Interestingly, in a number of brain pathologies PG levels have been reported to increase, suggesting that, in those conditions, the PG-dependent glutamate release from astrocytes could be dysregulated and participate in neurotoxic cascades.

In view of these important findings obtained in culture, it was imperative for us to establish whether the same mechanisms operate in astrocytes in situ. To address this possibility we started a collaboration with G. Carmignoto, L. Pasti and T. Pozzan at the University of Padua, working on acute hippocampal slices. We found that, in conditions where neuronal synaptic release in the slices was abolished [17], coactivation of mGluRs and AMPARs as well as direct PGE_2 application induced a release of glutamate sensitive to the Ca^{2+} chelator BAPTA/AM and to indomethacin, i.e. substantially identical to that observed in culture, and presumably originating from astrocytes.

We then addressed the physiological relevance of the glutamate release from astrocytes by imaging $[Ca^{2+}]_i$ changes in the CA1 region of the hippocampal slice with confocal microscopy [17]. In slices with inhibited neuronal exocytosis, application of PGE_2 induced $[Ca^{2+}]_i$ elevations in both astrocytes and pyramidal neurons. However, the neuronal $[Ca^{2+}]_i$ rises followed the astrocyte ones and were only indirectly caused by PGE_2 through glutamate release from astrocytes. In fact, when PGE_2 was administered in the presence of ionotropic GluR antagonists APV and NBQX, $[Ca^{2+}]_i$ elevations in several neurons were abolished, whereas astrocyte $[Ca^{2+}]_i$ responses were mostly unaffected [25].

These results provide the first demonstration in situ that receptor-mediated glutamate release from astrocytes acts as a signalling system to produce functional changes in the surrounding neurons. Altogether, our data outline the existence of a continuous bidirectional and highly integrated exchange of glutamatergic signals between neurons and astrocytes during synaptic activity. Thus, when releasing glutamate, presynaptic terminals signal not only to postsynapses but also to perisynaptic astrocytes, which can reply via glutamate

release and themselves induce $[Ca^{2+}]_i$ changes in the same or different neurons.

Do astrocytes release glutamate via regulated exocytosis?

The finding that astrocytes release glutamate in response to physiological stimuli, i.e. activation of membrane receptors by synaptically released neurotransmitters, and to do so they use a complex Ca^{2+}-dependent process totally distinct from the Ca^{2+}-independent phenomena observed in pathological conditions, suggests that these cells might possess a specific 'releasable pool' of transmitter possibly stored into a vesicular apparatus. Although 'terminal-like' specializations with organized active zones have not been reported in the ultrastructural studies performed to date on astrocytes, at least three different lines of evidence support an 'exocytosis hypothesis' of the ligand-dependent release from astrocytes and encourage further studies into its molecular and ultrastructural details: (i) cultured astrocytes express a large variety of the proteins forming the exocytosis fusion complex in presynaptic terminals [30–32]. As revealed by immunoelectron microscopical analysis, proteins such as synaptobrevin II and synaptophysin are selectively localized at the membrane of vesicular organelles [31]. Secretogranin II, a typical marker for regulated secretory granules, is also expressed in astrocytes, into a population of dense-core vesicles, from where it is released in response to secretagogues which cause $[Ca^{2+}]_i$ elevations [33]; (ii) α-latrotoxin, a component of black widow spider venom known to stimulate vesicular release from nerve terminals [34], induces sustained release of glutamate from cultured astrocytes with features identical to those observed in neurons [35]; (iii) our own data indicate that pretreatment of astrocytes with tetanus neurotoxin, which cleaves synaptobrevin and thereby blocks exocytosis in neurons, slowly abolishes Ca^{2+}-dependent glutamate release in astrocytes without affecting the Ca^{2+}-independent component [25]. Obviously, at present, alternative hypotheses for the Ca^{2+}-dependent release process of astrocytes cannot be ruled out, such as the involvement of glutamate-permeant anion channels. Additional evidence is required to firmly establish the presence of an exocytotic process. Indeed, ultrastructural studies should directly localize transmitter into vesicles, while functional studies should record membrane fusion and transmitter quantal events during the release process. Such studies are in progress, and we will likely know more details of the regulated transmitter release cascade of astrocytes in the near future.

In conclusion, although incapable of propagating action potentials, astrocytes seem to possess their own form of excitability, based on a sophisticated calcium coding that responds to the activation of surface glutamate receptors with regulated transmitter release.

Spatial propagation of calcium signalling in the astrocytic network

Gap-junction channels couple contacting astrocytes and allow them to function as a syncytium. Ions, small organic molecules like ATP and second messengers such as IP3 can move from cytoplasm to cytoplasm thanks to gap junctional coupling (for reviews see [36–38]). In 1997, C. Gianme and co-workers [39] showed that Ca^{2+} waves induced in cultured astrocytes need integral IP_3-sensitive calcium stores and functional gap-junction channels to propagate. More recently, additional components have been implicated in the mechanism of calcium wave propagation, such as the cytoskeletal configuration of astrocytes and an extracellular pathway mediated by released ATP [40, 41].

Whatever the mechanism, this feature of astrocytes is probably of outstanding physiological importance: indeed, in principle, an individual astrocyte activated during synaptic transmission can generate (i) a local response by elevating its $[Ca^{2+}]_i$ and releasing glutamate directly back to the original synaptic input or to adjacent ones, but also (ii) a distal response by propagating its initial $[Ca^{2+}]_i$ elevation to other coupled astrocytes, which might in this way be coordinated to release transmitter and affect synapses located at a distance from the original stimulus. In this latter case, astrocytes could function as temporal-spatial integrators of synaptic activity and create functional macrodomains in the brain circuitry (fig. 3). Whether astrocytes will respond to neuronal activity locally or distally might depend on the level of the neuronal activity itself and on the corresponding type of calcium signalling triggered in astrocytes (see above). In any case, propagation of calcium waves through the astrocyte network is clearly distinct from propagation of electrical signals along conducting axons in neurons, both in terms of spatial characteristics and of time scale (i.e. it is by far slower), and therefore its potential role in integration of synaptic function is clearly of a different type.

Signal propagation through the astrocytic network can have pathophysiological in addition to physiological implications. Indeed, during cerebral ischemia, astrocytes might facilitate spreading of injury from the core of the ischemic region to the bordering areas (the so-called 'penumbra'). In this light, M. Nedergaard and co-workers [42] have recently observed that oxygen deprivation reduces, but does not abolish, gap-junction

permeability, and astrocytes become totally uncoupled only when they are irreversibly committed to die. Since they die in groups rather than randomly isolated, the authors suggested that dying astrocytes might recruit potentially viable ones by spread of death signals through the open gap junctions. To prove this hypothesis, they generated glioma cell lines overexpressing the antiapoptotic gene bcl2 and resistant to lethal stimuli such as calcium overload, oxidative stress and metabolic inhibition. However, when these resistant cells were cultured with ordinary glioma cells or primary astrocytes and formed gap junctions with them, they lost most of their resistance. Death of resistant cells was strictly proportional to the degree of coupling with ordinary cells, indicating that activation of death pathways in more sensitive cells most likely leads to diffusion of proapoptotic factors overcoming bcl2 protection to the coupled cells via the maintained gap junctions [43].

Astrocytes modulate synaptic transmission

Increasing evidence suggests that astrocytes are critically involved in the proper formation of synaptic contacts during development as well as in the functional modulation of mature synapses. Studies in the developing retina indicate that a tight communication between neurons and astrocytes based on growth factor signals is essential to achieve regular axonal growth and synapse formation [44–46]. In addition, Pfrieger and Barres found that retinal neurons in cultures poor of astrocytes make less and less efficient connections than neurons with an astrocyte-reach environment [47]. The astrocyte modulation of ongoing synaptic activity has been very recently demonstrated both in culture and in situ. Using mixed cultures of hippocampal astrocytes and neurons, P. Haydon and co-workers [48] observed that increasing $[Ca^{2+}]_i$ in astrocytes specifically results in a number of changes in neuronal electrical properties and synaptic functions. First of all, astrocyte activation gives rise to slow inward currents in neighbouring neurons. Such currents are abolished by blockers of ionotropic GluRs, consistent with the evidence that $[Ca^{2+}]_i$ elevation in astrocytes promotes glutamate release, which activates neuronal GluRs [23, 25]. Second, coincident activation of astrocytes during evoked synaptic transmission reduces the amplitude of the postsynaptic currents at both inhibitory and excitatory synapses. This phenomenon is blocked by mGluR antagonists, and might result from activation of inhibitory presynaptic mGluRs by the astrocyte-released glutamate. Third, astrocyte stimulation increases the frequency of miniature postsynaptic currents (i.e. the currents evoked by spontaneous release of neurotransmitter) at both inhibitory and excitatory synapses. Unlike the effect on the evoked currents (mediated by mGluRs), this effect is sensitive to blockers of NMDARs, which the authors propose to be extrasynaptic [49].

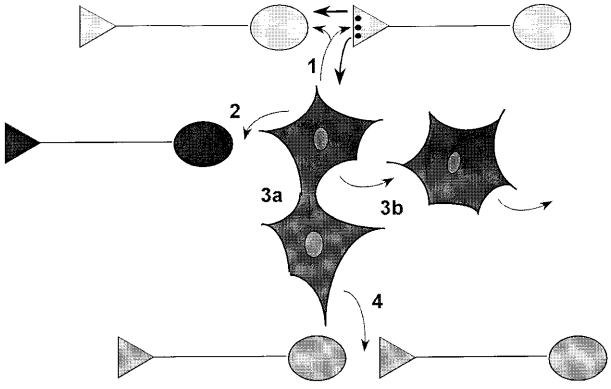

Figure 3. Possible pathways for synaptic signalling integration by astrocytes. From the top to the bottom: glutamate secreted from an active synapse induces $[Ca^{2+}]_i$ increase in a surrounding astrocyte leading to glutamate liberation. The following possibilities are then envisaged: (1) the astrocyte signals back to the original synapse, activating GluRs either pre-, post- or extrasynaptically. In addition (or in alternative), the activated astrocyte could (2) stimulate GluRs on a different neighbouring neuron; or (3a) propagate calcium waves via gap junctions to contacting astrocytes, inducing glutamate release at synapses located far from the starting signal (4); or (3b) activate GluRs located on a nearby, not contacting astrocyte, thus promoting 'paracrine' secretion of transmitter and starting new pathways for the travelling signal.

These initial observations not only confirm that astrocytic inputs can modify synaptic transmission, but also outline a potential complexity of the astrocyte modulation. Depending on a number of factors, including the type of ongoing activity, the characteristics of the neurons and astrocytes involved, their reciprocal morphological relations, the receptors expressed by the two cell types at relevant loci, glutamate release from the astrocytes may have distinct facilitatory or inhibitory effects on synaptic transmission. Three recent studies in situ confirm and strengthen the observations in vitro [19, 50, 51]. In a first study [50], a glial contribution to adaptive synaptic changes was revealed at the frog neuromuscular junction, a peripheral preparation in which Schwann cells respond to neuroligand stimulation similarly to astrocytes in the CNS [52]. High-frequency stimulation of the neuronal afferents activated perisynaptic Schwann cells while inducing synaptic depression at the neuromuscular junction. By stimulating or blocking G-protein-dependent signal-transduction specifically into the Schwann cells (via injection of nonhydrolizable guanosine triphosphate (GTP) and guanosine diphosphate (GDP) analogues) R. Robitaille observed modulation of synaptic depression and concluded that

activation of a signalling loop through the Schwann cells is a necessary mediatory component of the synaptic change. A second study performed in the rat eyecup preparation [51] found that initiation of $[Ca^{2+}]_i$ waves in the astrocytes produced a decrease in the firing rate of adjacent ganglion neurons in response to light. Astrocytes apparently act by releasing glutamate because GluR antagonists block the effect. This glutamate would depolarize the neighbouring GABAergic interneurons, which in turn inhibit the ganglion cells. A third study in acute hippocampal slices revealed a role for astrocytes in the activity-dependent potentiation of inhibitory synaptic transmission induced by repetitive firing of GABAergic interneurons [19]. In particular, firing of the GABAergic interneurons was accompanied by $[Ca^{2+}]_i$ elevations in astrocytes mediated by $GABA_B$ receptors. Generation of calcium waves in astrocytes by itself reproduced potentiation of synaptic activity, an effect blocked by antagonists of ionotropic GluRs, and therefore dependent on glutamate release from the astrocytes. Changes in the extracellular glutamate levels have been shown to play important roles in inhibitory transmission by activating kainate-selective receptors such as GluR5 [53], which are expressed on hippocampal interneurons and, upon stimulation, cause repetitive action potential firing of inhibitory fibers [54, 55]. This might be the mechanism underlying astrocyte potentiation of inhibitory transmission. The observation that GABA, in addition to glutamate, enhances $[Ca^{2+}]_i$ in astrocytes and starts back signalling to neurons, indicates that astrocyte activation during synaptic transmission is a phenomenon not restricted to glutamatergic synapses.

Do astrocytes possess independent 'microdomains'?

The acquisition that neuron-astrocyte circuits participate in signal elaboration at synapses stimulates new critical questions, e.g. whether transmitter signalling between the two cell types occurs in a nonspecialized, paracrine-like fashion, mainly regulated by diffusion laws or, instead, involves some level of input/output specificity. A very recent study by the group of H. Kettenmann [56] would favour the latter possibility. The researchers conducted experiments on cerebellar slices to better elucidate the morphological and functional features of the Bergmann glia. A three-dimensional computer reconstruction revealed that organization of these cells is not totally irregular. In particular, the authors noticed a high number of fine ramifications, formed by branching extensions all departing from a radial fiber. Each ramification formed a unit composed by a thin, long stalk ending with a 'cabbagelike head', which they called a glial microdomain. Each of these structures seems to be metabolically independent since it contains a number of mitochondria and ensheaths one or more synapses. Very interestingly, electrical stimulation of neuronal parallel fibers caused localized $[Ca^{2+}]_i$ increases in the Bergmann glia, restricted to small compartments of less than 100 μm^2, approximately corresponding to the size of an anatomically defined microdomain. The authors thus arrived at the remarkable conclusion that a Bergmann glial cell may consist of hundreds of independent compartments capable of autonomous interactions with the particular group of synapses they ensheath.

These findings add further complexity to the physiology of astrocytes, in which very specialized local functions and syncytial properties apparently coexist.

Conclusions

The recent findings reviewed here provide a new and exciting view of the role of astrocytes in brain function. Their unexpected level of integration with neurons and their active participation in synaptic processes are dense with implications, and so is the finding that they possess their own, nonelectrical, calcium-dependent form of excitability, able to trigger regulated transmitter release in response to synaptic inputs. Indeed, such release from astrocytes may be involved in a number of critical physiological functions, including synaptic transmission, modulation and plasticity as well as developmental regulation. As a consequence, dysregulation of the astrocyte pathways may have deep and previously unsuspected impacts on neuronal function and survival. Appreciation that, although anatomically and physiologically profoundly different from neurons, astrocytes play an active role in the processing of brain information, calls for attention to these cells. We are certainly only at the beginning of understanding their specific physiological properties, functional roles and relations with the neuronal partners; however the recent breakthroughs demonstrate that we cannot fully understand brain processes in health and disease without extending our refined knowledge to glial cells.

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