Communication between astrocytes and neurons: a complex language

Gertrudis Perea, Alfonso Araque*
Instituto Cajal, Consejo Superior de Investigaciones Científicas, Madrid 28002, Spain

Abstract

In recent years, accumulating evidence suggests the existence of bidirectional communication between astrocytes and neurons, indicating an important active role of astrocytes in the physiology of the nervous system. As a consequence of this evidence, a new concept of the synaptic physiology—"the tripartite synapse"—has been proposed, in which the synapse is formed by three functional elements, i.e. the pre- and postsynaptic elements and the surrounding astrocytes. In the present article we review and discuss the current knowledge on the cellular mechanisms and physiological properties of this communication that displays highly complex characteristics. We are beginning to realize that the communication between astrocytes and neurons uses a quite complex language.

Keywords: Astrocyte-neuron signaling; Intracellular calcium; Glutamate release; Synaptic modulation; Brain slices

1. Introduction

Gliaal cells were originally described by Rudolf Virchow in 1846 [78] as non-neuronal cells constituting the "glue" of the brain. Later studies classified glial cells of central nervous system (CNS) into two groups, microglia and macroglia. Microglia are macrophage like-cells with phagocytic functions. Macroglia are composed of two types of cells: oligodendrocytes, responsible for the myelination in the CNS (like myelinating Schwann cells in the peripheral nervous system), and astrocytes. Among these cells types, astrocytes play critical roles in the development and physiology of the CNS, being involved in key aspects of neuronal function, such as trophic support [58,74], neuronal survival [57] and differentiation [72], neuronal guidance [36,56], neurite outgrowth [38,69], and synaptic efficacy [39,53]. Furthermore, astrocytes contribute to brain homeostasis, regulating the local concentrations of ions [37,47] and neuroactive substances [9,37,41].

Since the late 1980s, the application of newly developed and refined cellular and physiological techniques (such as patch-clamp, ion-sensitive fluorescence imaging, confocal microscopy and molecular biology) to glial studies has challenged the classical idea that astrocytes simply provide structural and trophic support for neurons, suggesting that astrocytes play more active roles in the physiology of the CNS.

In the present article we will review the evidence showing the properties and cellular mechanisms of the signaling between astrocytes and neurons, specially focusing on studies performed in brain slices. The important metabolic, structural and homeostatic functions of astrocytes have been extensively reviewed (see references above) and will not be further discussed.

2. Excitability of astrocytes is based on intracellular Ca$^{2+}$ variations

Astrocytes were classically considered as non-excitable cells because they do not show electrical excitability. Indeed, although astrocytes can express voltage-gated channels [8,70], astrocytic membrane potential is relatively stable. Pioneering electrophysiological studies demonstrated that astrocytes exhibit little membrane potential variations and only responded passively to neuronal activity by sensing the extracellular potassium concentration [47].

However, the development of methods to study intracellular ions in living cells have allowed the demonstration that astrocytes display a form of excitability based on intracellular Ca$^{2+}$ variations [15,19]. A great effort is currently being made to define the mechanisms underlying this form of excitability, as well as its properties and functional significance.
3. Intracellular and intercellular Ca\(^{2+}\) waves in astrocytes

Ca\(^{2+}\) elevations in astrocytes may serve as an intra and intercellular signal that can propagate within and between astrocytes, signaling to different regions of the cell and to different cells.

3.1. Intercellular waves

In cell culture, Ca\(^{2+}\) elevations in astrocytes can propagate nondecrementally to adjacent astrocytes, forming a Ca\(^{2+}\) wave that can extend for hundreds of micrometers [15,19,30]. Several studies have suggested that Ca\(^{2+}\) waves are propagated by diffusion of inositol triphosphate across gap junctions, since they are prevented by gap junction blockers [23,75]. Furthermore, glial cell lines, which do not form gap junctions and do not exhibit Ca\(^{2+}\) waves, do present Ca\(^{2+}\) waves after ectopic expression of the gap junction proteins, connexins [20]. However, other studies suggest that the intercellular Ca\(^{2+}\) waves are mediated by an extracellular signal. Accumulating evidence show that astrocytic Ca\(^{2+}\) waves are mediated by extracellular ATP and subsequent activation of purinergic receptors [16,25]. Indeed, Ca\(^{2+}\) waves can propagate across cell-free gaps between cells [28], waves are still present in cultured astrocytes of connexin 43 knock-out mice [63], and purine receptor antagonists or apyrase (an ATPase) reduce Ca\(^{2+}\) wave propagation [16,25]. Moreover, it has been demonstrated that ATP can be released from astrocytes during a Ca\(^{2+}\) wave [16,25,30]. Taken together, these results suggest that ATP is the extracellular signal involved in the Ca\(^{2+}\) wave propagation. In addition to ATP, as the main extracellular signal involved in Ca\(^{2+}\) wave propagation, nitric oxide may also regulate Ca\(^{2+}\) waves [79].

Although the hypotheses that Ca\(^{2+}\) waves are mediated by gap junctions or by ATP as extracellular signal seem contradictory, it is noteworthy that they are not mutually exclusive. Furthermore, both interpretations can be reconciled by recent data showing that ectopic expression of connexins leads to an increase in both ATP release and the radius of Ca\(^{2+}\) wave propagation, which suggest that connexins can mediate ATP release through unknown mechanisms [17].

While intercellular Ca\(^{2+}\) waves have been undoubtedly demonstrated in cultured astrocytes, the presence of Ca\(^{2+}\) waves in acute brain slices is still controversial. Astrocytic Ca\(^{2+}\) waves have been demonstrated in the retina and in organotypic cultures of hippocampal slices [21,26,45], but they were not originally detected in acute hippocampal slices [51,54]. However Ca\(^{2+}\) waves have been recently reported in acute brain slices [64,71].

3.2. Intracellular waves

Ca\(^{2+}\) elevations in astrocytes can be spatially restricted to localized regions of the cell. In hippocampal slices, stimulation of Schaffer collaterals induces Ca\(^{2+}\) elevations in restricted regions of the astrocyte that eventually can propagate throughout the processes of the cell [14,51]. Likewise, in cerebellar slices, Bergmann glial cells, a specialized type of cerebellar astrocytes, show sub-cellular microdomains that respond independently to stimulation of parallel fibers [24].

The existence of localized sites of Ca\(^{2+}\) elevations is not exclusive of the astrocytic responses to neuronal activity. Indeed, spontaneous astrocytic Ca\(^{2+}\) oscillations, which are independent of neuronal activity (see below), arise within discrete regions of astrocytic processes, and can eventually propagate along cell processes [44,50].

The compartmentalization of the Ca\(^{2+}\) signal and the eventual propagation to different regions of the cell may be extremely important in the physiology of the astrocytes and their interactions with neurons, and it will be discussed in more detail below.

3.3. Spontaneous oscillations

Parri et al. [50] using slices of rat ventrobasal (VB) thalamus, and Nett et al. [44] using hippocampal slices have demonstrated that astrocytes may exhibit spontaneous Ca\(^{2+}\) oscillations that are independent of neuronal activity. Pharmacological experiments indicate that they are mediated by intracellular Ca\(^{2+}\) release because they are abolished after depletion of intracellular Ca\(^{2+}\) stores. The role of these spontaneous Ca\(^{2+}\) oscillations is an exciting open question. Nevertheless, since they are independent of neuronal activity and can modulate neuronal excitability, they may serve as an independent source for generation and modulation of neuronal activity. Interestingly, the occurrence of spontaneous oscillations in VB thalamus is clearly age-dependent, suggesting the possible participation of these oscillations in the development [50].

Furthermore, a recent report [73] has shown that in cortical slices in which epileptic conditions were pharmacologically induced, astrocytes exhibit Ca\(^{2+}\) oscillations that are independent of the neuronal activity and the epileptiform discharges. It is unknown however whether these oscillations are contributing to the epileptiform activity or are a consequence of it.

4. Astrocytic Ca\(^{2+}\) elevations can be triggered by synaptically-released neurotransmitters

Astrocytes express a wide variety of functional receptors for many neurotransmitters (for a detailed review, see Ref. [55]). In addition to ionotropic receptors, many
of the neurotransmitter receptors expressed by astrocytes are metabotropic, being coupled to different second intracellular messenger pathways that can lead to different intracellular regulatory responses [33, 42]. Activation of some of these receptors by exogenous application of neurotransmitters, including glutamate, adenosine, norepinephrine, GABA, histamine, and acetylcholine (ACh) can increase the intracellular Ca^{2+} concentration both in vitro and in situ [7, 11, 22, 32, 35, 51, 54, 55, 61, 65–67, 77].

Histological studies have shown that astrocytic processes are intimately associated with synapses in many brain regions [59, 60, 76], which potentially provides a suitable physical arrangement for the existence of signaling between synapses and astrocytes. However, whether the astrocytic neurotransmitter receptors can be activated by synaptically-released neurotransmitters is not fully determined. Indeed, activation of astrocytic receptors by neurotransmitters released from synaptic terminals has been so far demonstrated for only a relatively low number of neurotransmitters and in a few brain regions. In the following paragraphs, a brief description of the currently reported astrocytic responses to synaptically-released neurotransmitters in brain slices will be summarized.

Stimulation of glutamatergic Schaffer collaterals in hippocampal slices evokes Ca^{2+} increases in astrocytes located in the stratum radiatum of the CA1 area [11, 21, 51]. These Ca^{2+} elevations are mediated by synaptically-released glutamate acting on metabotropic glutamate receptors (mGluRs), because they are sensitive to tetrodotoxin and 4-aminopyridine, and abolished by the mGluR antagonist MCPG [51, 54] and mimicked by the mGluR agonist r-ACPD [51].

Kang et al. [32] have shown GABA-mediated astrocytic Ca^{2+} elevations in hippocampal slices. Direct depolarization of patch-clamped interneurons evokes Ca^{2+} elevations in astrocytes that are prevented by GABA B receptor antagonists. Analysis of the spatial relationship between the patch-clamped neuron and the responding astrocyte demonstrate that the axon of the stimulated interneuron was ensheathed in processes of the astrocyte.

We have recently demonstrated in hippocampal slices that stimulation of stratum oriens/alveus of the hippocampus, which contains cholinergic and glutamatergic axons, evokes long-lasting inward currents and transient, long-lasting Ca^{2+} elevations in astrocytes located in the stratum oriens of the CA1 region [7]. Both astrocytic responses are abolished by tetrodotoxin or Cd^{2+}, and are increased by 4-aminopyridine, indicating that the responses are mediated by synaptically-released neurotransmitter. The inward current is inhibited by glutamate transporter antagonists, indicating that it is due to the activity of electrogenic glutamate transporters. The synthetically-evoked intracellular Ca^{2+} elevations are unaffected by glutamate receptor antagonists, but are abolished by atropine, indicating that they are mediated by muscarinic cholinergic receptors (mAChRs). Thapsigargin prevents the Ca^{2+} elevation but does not modify the inward current, indicating that the Ca^{2+} signal is due to intracellular Ca^{2+} mobilization [7]. These results indicate that hippocampal astrocytes can respond to synaptically-released acetylcholine through activation of mAChRs, that can mobilize Ca^{2+} from the intracellular stores.

Kulik et al. [35] have shown in cerebellar slices that Bergmann glial cells respond to norepinephrine. High frequency stimulation within the molecular or granule cell layer leads to Ca^{2+} elevation in Bergmann glial cells due to synaptically-released norepinephrine. A detailed pharmacological study of these responses indicates that they are not mediated by α2- or β-adrenoceptors, but they are selectively mediated by activation of α1-adrenoceptors.

Using the same preparation, Matyash et al. [40] have recently reported that parallel fiber stimulation also induces Ca^{2+} elevations in Bergmann glial cells. The Ca^{2+} increases are not mediated by glutamatergic receptors because they are insensitive to ionotropic or metabotropic glutamate receptor antagonists, but they are prevented by nitric oxide-synthase inhibitors, suggesting that they are mediated by nitric oxide (NO).

Neuron-glia interaction is not exclusive of astrocytes because presynaptic Schwann cells in the peripheral nervous system respond with Ca^{2+} elevations to neurotransmitter release [31, 61, 62]. Furthermore, Bergles et al. [10] have recently shown that Schaffer collateral stimulation evokes AMPA receptor-mediated currents in stratum radiatum oligodendrocyte precursor cells.

Understanding the mechanisms and properties of the neuron-evoked astrocytic Ca^{2+} elevations is extremely relevant because its potentially important consequences in neuronal physiology (see below). In the following paragraphs we will summarize the most remarkable characteristics of the astrocytic responses to the neuronal activity described so far.

5. Astrocytic responses depend on the level and pattern of synaptic activity

Schaffer collateral stimulation in hippocampal slices causes Ca^{2+} elevations in stratum radiatum astrocytes that are dependent on the frequency of the stimulation. While low frequencies fail to evoke astrocytic responses, stimulation at high frequencies evokes Ca^{2+} elevations. Furthermore, under continuous stimulation these Ca^{2+} elevations become oscillatory, and the frequency of the oscillations changes according to the firing rate of neuronal afferents [51]. Moreover, Pasti et al. also showed that an increase in the intensity of the stimulus results in a clear increase in the frequency of the Ca^{2+} oscilla-
tions. The important functional meaning of these relationships become clearer after the demonstration by these authors that the frequency of the Ca$^{2+}$ oscillations may represent the code that controls the glutamate release from astrocytes [52].

Likewise, we have recently shown that electrical stimulation of the stratum oriens/alveus of the hippocampus evokes a long-lasting inward current and intracellular Ca$^{2+}$ elevations in stratum oriens astrocytes, and that these responses depended on both the frequency and duration of the stimulus [7].

Synaptically-induced GABA-mediated Ca$^{2+}$ responses in hippocampal astrocytes also depend upon neuronal firing pattern [32].

Studies performed in cerebellar slices also show that synaptically-mediated Ca$^{2+}$ elevations in Bergmann glial cells depend on the degree of synaptic activity. Kulik et al. [35] have demonstrated that the amplitude of the norepinephrine-mediated Ca$^{2+}$ transients evoked by tetanic stimulation within the granule cell layer depends on the frequency, strength, and duration of the stimulus. Furthermore, in the same preparation, the amplitude of the astrocytic Ca$^{2+}$ responses evoked by parallel fiber stimulation varied with the stimulation frequency [40].

Taken together, these results suggest that the astrocytes are able to discriminate between different levels and patterns of synaptic activity.

6. Plasticity of the synaptically-evoked astrocytic Ca$^{2+}$ signal

Pasti et al. [51] have shown that the frequency of astrocytic Ca$^{2+}$ oscillations can be modified by previous activity. These authors have demonstrated that the oscillatory frequency of astrocytic Ca$^{2+}$ may increase after repetitive challenges with the mGluR agonist t-ACPD or repetitive stimulation of glutamatergic Schaffer collaterals, indicating that astrocytic responses can be modified by previous episodes of synaptic activity [51].

7. Astrocytes respond to synaptic terminals from extrinsic pathways

As described above, it is now well established that astrocytes can be activated by neurotransmitters released from immediately adjacent synapses formed by axon terminals of neurons belonging to the local circuit in which the astrocytes are immersed (for a review, see Ref. [4]). However, may astrocytes also respond to extrinsic axons arising from a different brain area? To address this question we have investigated whether an extrinsic cholinergic pathway to the hippocampus can signal to astrocytes regulating their intracellular Ca$^{2+}$. Using electrophysiological and single-cell fluorescence photometric Ca$^{2+}$ techniques in hippocampal slices, we have found that stimulation of the stratum oriens/alveus of the hippocampus, which contains cholinergic afferents from the septum and diagonal band of Broca, increases the intracellular Ca$^{2+}$ of astrocytes in the hippocampal stratum oriens through activation of mACHRs [7]. These results demonstrate that astrocytes are a target of extrinsic axons arising from a different brain area, which adds further complexity to the signaling pathways in the CNS.

8. Sub-cellular microdomains in astrocytes respond independently to synaptic activity

Ultrastructural studies and three-dimensional reconstruction of Bergmann glial cell processes have revealed subcellular compartments called microdomains that have a complex surface consisting of thin membrane sheets that wraps synapses between parallel fiber axon terminals and Purkinje neuron spines [24]. These authors have also investigated the Ca$^{2+}$ responsiveness of these microdomains to synaptic activity in cerebellar slices. They have found that not all processes from a given cell respond to stimulation of parallel fibers, rather, only small areas of the cell exhibited Ca$^{2+}$ elevations. Moreover, the small compartments within cell processes that respond to synaptic activity are similar in size to structurally observed microdomains [24].

Like Bergmann glial cells, Ca$^{2+}$ signalling can also be spatially restricted to discrete sites in hippocampal astrocytes. A detailed analysis of the Ca$^{2+}$ signal evoked in hippocampal stratum radiatum astrocytes in response to Schaffer collateral stimulation has revealed that multiple and spatially distinct astrocytic processes show Ca$^{2+}$ elevations that are not synchronized, suggesting that the synaptically-evoked Ca$^{2+}$ signaling can be independently compartmentalized in different astrocytic processes [14, 51]. Recently, Nett et al. [44] have also confirmed the existence of microdomains in hippocampal astrocytes because the spontaneous astrocytic Ca$^{2+}$ increases can be confined to parts of a single process.

We have also found in hippocampal slices that synaptically-induced cholinergic-mediated Ca$^{2+}$ variations in stratum oriens astrocytes are spatially defined. While some astrocytic processes increase their intracellular Ca$^{2+}$ levels, other regions fail to respond to axonal stimulation. Therefore, sub-cellular regions of astrocytes may respond differentially to the synaptic cholinergic activity [7].

Taken together, these results suggest that Bergmann glial cells and astrocytes may consist of hundreds of independent compartments capable of autonomous interactions with the particular group of synapses that they ensheath.

The mechanisms controlling whether the Ca$^{2+}$ signaling remains locally restricted to each process or
extends along and to adjacent process may be of great significance, because they will determine the spread of the intracellular signal that triggers glutamate release (see below) modulating adjacent synapses. Indeed, histological studies suggest that adult rat hippocampal CA1 stratum radiatum astrocytes establish separate anatomical domains in the neuropil [12], occupying an averaged neuropilar volume of ~66,000 μm³. Considering that in this hippocampal region there are ~213 synapses/μm³ [34], a single astrocyte could eventually influence ~140,000 synapses [12]. Thus, a single astrocyte can cover a large number of synapses that can be differentially influenced by a single astrocyte according to the degree of extension of the intracellular astrocytic Ca²⁺ signal.

9. Astrocytic functional domains discriminate between synaptically-released neurotransmitters

As described above, stimulation of parallel fibers, which uses glutamate as the main neurotransmitter, evokes NO-mediated responses in Bergmann glia [40]. Co-stimulation of cholinergic and glutamatergic axons evokes cholinergic- but not glutamatergic-mediated Ca²⁺ elevations in hippocampal astrocytes [7]. Therefore, although astrocytes express different types of neurotransmitter receptors, these are selectively activated, indicating that the ability of astrocytes to respond to different neurotransmitters is precisely controlled. How this selective discrimination is achieved remains unknown. However, we have hypothesized a restricted spatial localization of the receptors [7] (see below).

Furthermore, our studies on the responses of stratum oriens astrocytes to synaptic activity in hippocampal slices have revealed the capacity of astrocytes to exhibit discriminated responses to different neurotransmitters, suggesting the existence of functional domains in astrocytes [7]. Using simultaneous electrophysiological and single-cell Ca²⁺ measurements in hippocampal slices (Fig. 1), we have found that electrical stimulation of the stratum oriens/aleveus, which contains cholinergic afferents form the septum and diagonal and of Broca as well as glutamatergic axons from CA1 pyramidal neurons, evokes long-lasting inward currents and Ca²⁺ elevations in stratum oriens astrocytes [7] (Fig. 1). We demonstrated pharmacologically that both astrocytic responses, the inward current and the Ca²⁺ elevation, are mediated by synaptically-released neurotransmitters and they are due to different and independent mechanisms. While the astrocytic inward current is mediated by activation of glutamate transporters, the Ca²⁺ increase is mediated by activation of mACHRs [7].

Stimulation of glutamatergic Schaffer collaterals evokes Ca²⁺ elevations in stratum radiatum astrocytes [11,51,54] through activation of mGluRs. Contrastingly, stimulation of stratum oriens/aleveus evokes Ca²⁺ elevations in stratum oriens astrocytes that are mediated by cholinergic rather than glutamatergic receptors, because they are abolished by atropine but are insensitive to CNQX, D-AP5 or MCPG. The lack of glutamate effect on these neuron-evoked astrocytic Ca²⁺ signaling is not due to the absence of functional glutamate receptors because ionophoretically-applied glutamate increases the astrocytic Ca²⁺ levels. Furthermore, the recorded glutamate transporter currents demon-
strate that astrocytes are able to sense synaptically-released glutamate [7]. Therefore, although astrocytes express functional glutamate receptors and astrocytes are exposed to synaptically-released glutamate, astrocytic glutamate receptors may be spared from the glutamate released by these specific synapses.

Can these glutamate receptors be activated by synaptically-released glutamate? One could argue that these glutamate receptors would be activated by this axon pathway under different conditions. Alternatively, it could be hypothesized that these receptors can be activated by a different glutamatergic pathway. To test this latter hypothesis, we have stimulated Schaffer collateral afferents, which are the main glutamatergic input to the CA1 hippocampal region, while recording electrophysiological and intracellular Ca2+ responses in stratum oriens astrocytes. We have found that stimulation of this axonal pathway evokes intracellular Ca2+ elevations that are mediated by activation of astrocytic glutamate receptors (our unpublished results).

Taken together, these results suggest the existence of functional astrocytic sub-cellular domains that may respond independently to different synaptically-released neurotransmitters, and that astrocytes may discriminate between the activity of different synaptic terminals belonging to different axon pathways.

Furthermore, these results indicate that while different neurotransmitters are release by relatively close synaptic terminals, astrocytes may discriminate between them. It can be therefore hypothesized that functional astrocytic receptors are spatially inserted in the membrane under precise control, allowing their selective activation by specific synaptic neurotransmitters.

**10. Ca2+ elevations in astrocytes modulate neuronal excitability and synaptic transmission**

Several groups have demonstrated that astrocytes not only respond to synaptically-released neurotransmitters with intracellular Ca2+ elevations, but in addition this Ca2+ signal may in turn trigger a feedback signal to neurons.

Using different stimuli that elevate intracellular Ca2+ in astrocytes, cell culture studies have shown that Ca2+ elevations in astrocytes can lead to glutamate-dependent Ca2+ elevations in adjacent neurons [18,27,43,48]. These initial cell culture studies were later confirmed and extended in hippocampal slices [11,32,51]. Studies on the cellular mechanism involved in this glutamate release from astrocytes have shown that it is a Ca2+-dependent process that requires the presence of functional vesicle-associated proteins, suggesting that astrocytes release glutamate through an exocytotic pathway [1–3,5,13,52].

The glutamate-mediated astrocyte-to-neuron signal is also manifested electrophysiologically by neurons. Using mixed cultures of rat hippocampal astrocytes and neurons, different stimuli that elevate intracellular Ca2+ in astrocytes caused a glutamate-dependent slow inward current (SIC) in adjacent neurons [1–3,5,49]. In current-clamp recordings, astrocytic Ca2+ elevations can induce membrane depolarization in neurons that eventually can trigger action potential discharges [3,27]. The presence of astrocyte-induced slow inward currents in neurons has also been recently reported in acute slices of the rat ventrobasal thalamus [50]. These authors have demonstrated that astrocytic Ca2+ oscillations spontaneously occurring in ventrobasal thalamus slices induces slow inward currents in thalamocortical neurons. These neuronal inward currents, which could depolarize the neuronal membrane potential beyond the action potential firing threshold, correlate with the Ca2+ elevations and propagating waves in neighboring astrocytes, and are sensitive to the NMDA receptor (NMDAR) antagonist D-AP5, indicating that they are mediated by glutamate release from astrocytes [50].

Ca2+-dependent glutamate-mediated glial modulation of neuronal excitability was also demonstrated in the retina, where Ca2+ elevations in Müller cells and astrocytes can modulate the output of retinal ganglion cells in response to light activation of photoreceptors [46]. When the glial Ca2+ wave reaches the recorded ganglion cell, either an excitation or inhibition of ganglion cell activity is detected. The inhibition of the ganglion cell activity is abolished by glutamate and GABAA receptor antagonists. Newman and Zahs [46] suggested that glutamate is released from the glia onto amacrine cells which in turn release GABA and glycine to inhibit retinal ganglion cell activity. In addition to modulate neuronal excitability, Ca2+-dependent glutamate release from astrocytes can also regulate synaptic transmission. In cultured hippocampal cells, stimulation of astrocytes leads to a reduction of the amplitude of action potential-evoked excitatory and inhibitory postsynaptic currents through activation of presynaptic mGluRs, which are known to inhibit neurotransmitter release [1].

In the same preparation, Araque et al. [2] have described that astrocytic stimulation transiently increases the frequency of excitatory as well as inhibitory miniature postsynaptic currents (mPSCs), without modifying their amplitude distribution. Using pharmacological tools (thapsigargin, the Ca2+ chelator BAPTA, and photolysis of the Ca2+ cage NP-EGTA), these authors have demonstrated that elevation of intracellular Ca2+ in astrocytes is both necessary and sufficient to induce an increase in the frequency of miniature postsynaptic currents (mPSCs). This astrocytic-induced mPSC frequency increase is abolished by the NMDAR antagonist D-AP5, but it is not prevented by specific blockade of the postsynaptic NMDARs with the open channel blocker MK801, indicating that the mPSC frequency
increase is mediated by the activation of presynaptic NMDA receptors [2]. Kang et al. [32] have demonstrated the participation of astrocytes in the modulation of miniature inhibitory synaptic transmission in acute hippocampal slices. They have shown that depolarization of astrocytes or activation of GABAergic interneurons leads to Ca\(^{2+}\) elevations in astrocytes, and causes a glutamate-dependent increase of the frequency of miniature synaptic currents in pyramidal neurons [32].

Like neuron-to-glia signalling, glial modulation of synaptic activity is also present in peripheral nervous system. Robitaille’s group has elegantly demonstrated that perisynaptic Schwann cells can modulate synaptic transmission [61,62].

Furthermore, a novel Ca\(^{2+}\)-independent mechanism for astrocytic modulation of synaptic transmission has been recently reported in the molluscan CNS, where synaptically-released ACh induces the release of an ACh-binding protein from glial cells that modulate cholinergic transmission [68].

In summary, since Ca\(^{2+}\) elevations in astrocytes evoke the release of glutamate [1–3,5,11,49], which can modulate the neuronal activity and synaptic transmission [1–3,32,46], the neurotransmitter-mediated astrocytic Ca\(^{2+}\) increases may lead to glutamate release that modulate neuronal activity and synaptic transmission.

11. Concluding remarks

The results described in this review clearly demonstrate the existence of a complex bidirectional communication between glial cells and neurons and indicate an important active role of glial cells in the physiology of the nervous system [6,14,29]. Astrocytes possess a form of excitability based on intracellular Ca\(^{2+}\) variations that can be triggered by synaptically-released neurotransmitters. These Ca\(^{2+}\) elevations evoke Ca\(^{2+}\)-dependent glutamate release from astrocytes that can signal to adjacent neurons, leading to an astrocyte-induced glutamate-mediated modulation of the neuronal excitability and synaptic transmission. As a consequence of this feedback signaling between astrocytes and neurons, a new concept of the synaptic physiology has been proposed, in which the synapse is functionally formed by three elements, i.e. the pre- and postsynaptic elements and the surrounding astrocytes (for a review see Ref. [4]).

In conclusion, while a considerable amount of work is needed to thoroughly understand the properties and mechanisms of the bidirectional communication between astrocytes and neurons, a new vision of the physiology of the CNS has emerged, where astrocytes, by reciprocally exchanging information with neurons, play a more active role than previously thought. Moreover, considering the complex properties of this bidirectional communication that we are just starting to understand, we are beginning to appreciate the existence of new highly rich forms of communication in the CNS. As Haydon [29] has recently described, we now know that astrocytes and neurons talk to each other. As we are understanding the words of this communication (i.e. its elements), we are beginning to realize that this communication uses a quite complex language.

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