Chapter

Synaptic Functions of Astroglial Hemichannels

Juan A. Orellana

Abstract

In recent decades, astrocytes have gained ground in their protagonist role at the synapses, challenging the old-historic idea that neurons are the unique functional units in the nervous system. Although for a long time considered merely supportive elements, astrocytes are now recognized as a source of gliotransmitter release that regulates synaptic transmission and plasticity. Despite the initial evidence that supported gliotransmission depends on intracellular Ca^{2+}-mediated vesicular release, recent data indicate that hemichannels may constitute an alternative non-vesicular route for gliotransmitter efflux. These channels are plasma membrane channels formed by the oligomerization of six connexins around a central pore. Hemichannels are permeable to ions and signaling molecules—such as ATP, glutamate, and Ca^{2+}—constituting a pathway of diffusional interchange between the cytoplasm and the extracellular milieu. Connexin 43 is the main hemichannel-forming protein in astrocytes and is highly regulated under physiological and pathological conditions. In this chapter, the available data supporting the idea that hemichannels are chief components in tuning the synaptic gain in either resting or stimulated conditions is discussed.

Keywords: connexin 43, astrocyte, gliotransmission, brain, neuron

1. Introduction

In order to ensure a proper response to external stimuli, organisms have created complex and coordinated neural structures that allow the sophisticated analysis of information. As the central nervous system (CNS) evolved from a basic network structure to compacted ganglia and centralized brains, two types of connections emerged as specialized structures favoring the integration of neural networks [1]. In 1897, Sherrington proposed the point of functional contact between neurons as the specific area at which transfer of information takes place and named it "synapsis," soon shortened to the "synapse," from the Greek word *sunáptō* (to clasp) [2]. This specialized structure is known today as the chemical synapse and transfers electrical information unidirectionally from presynaptic to postsynaptic neurons through the release of neurotransmitters, which, acting upon postsynaptic receptors, initiate a second electrical signal [1]. In the late 1950s, Furshpan and Potter reported a series of experiments revealing that synaptic transmission in the crayfish is bidirectional and voltage-dependent, two properties substantially out of range of the criteria established for chemical transmission [3]. This study revealed the pioneer evidence in favor of the existence of electrical synaptic transmission. Unlike chemical synapse, the electrical synapse permits the bidirectional

flow of ions between coupled neurons that come markedly close at intercellular specializations called gap junctions [4] (**Figure 1**). Nowadays, a growing body of evidence indicates that both mechanisms of synaptic transmission—chemical and electrical—are complementary and highly intermodulated to ensure proper brain development and function [1].

The traditional notion of neurons being the only functional elements in the synapse has been questioned with the finding that intracellular Ca^{2+} ($[Ca^{2+}]_i$) waves within and among astrocytes underlie the regenerative (nondissipative) transfer of biological signals [5–7]. Although astrocytes are not electrically silent cells [8], $[Ca^{2+}]_i$ signals are their principal fast time-scale mechanism for allowing intra- and intercellular signaling [9]. These signals base their origin on the extracellular influx of Ca^{2+} via ion channels and through Ca^{2+} release from intracellular stores, resulting in $[Ca^{2+}]_i$ transients that differ in frequency, kinetics, and spatial spread depending on the astroglial anatomical region [10]. Endowed with this machinery and along with pre- and postsynaptic neuronal elements, astrocytes embrace the "tripartite synapse"—the Rosetta stone of the chemical synaptic transmission—in where they sense neurotransmission and respond to it by releasing biomolecules that regulate neuronal activity called "gliotransmitters" (i.e., glutamate, D-serine, and ATP) [11]. Intracellular $[Ca^{2+}]_i$ waves can spread among astrocytes to finally reach the terminal processes or "endfeet" of specialized astrocytes that contact the endothelium [12]. There, vasoactive molecules are released, permitting astrocytes to modulate the cerebral blood flow (CBF) and delivery of energy substances (i.e., glucose and lactate) with potentially significant consequences for neuronal firing and higher brain functions [13]. Indeed, a single astrocyte may contact over 100,000 synapses in rodents and up to 2,000,000 synapses in humans, revealing that they actually form a syncytium with multiple connections [14].

Nowadays, diverse mechanisms have been proposed to lead to gliotransmitter release (**Figure 1**), including Ca^{2+}-dependent exocytosis [15–17], carrier membrane transport [18], and opening of a wide range of channels. Among the latter group, volume-regulated anion channels [19–21], P2X7 receptors [22–24], Ca^{2+}-dependent Cl^- channel bestrophin 1 [25, 26], and hemichannels [27–30] are included. This

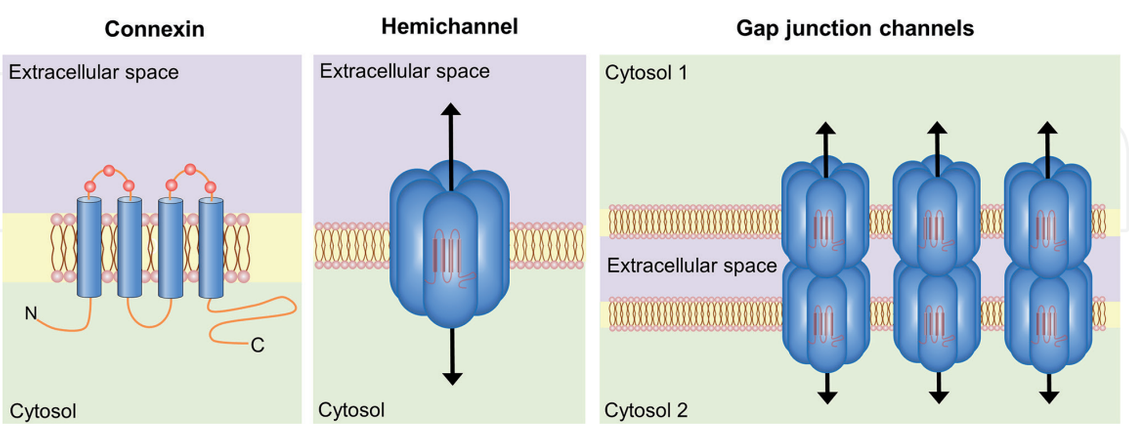

Figure 1.
Basic structure of connexin-based channels. Connexins have four α-helical transmembrane domains connected by two extracellular loops and one cytoplasmic loop; both the amino- and carboxy-termini are intracellular. The relative positions of the extracellular loop cysteines (red balls) are also shown. Hemichannels (also known as connexons) are formed by the oligomerization of six subunit connexins around a central pore. Under resting conditions, hemichannels remain preferentially closed, but they may be activated by diverse physiological and pathological conditions and offer a diffuse transmembrane route between the intra- and extracellular milieu. Hemichannels dock each other to form functional cell-to-cell channels termed gap junction channels (right panel). Gap junction channels aggregate in well-known anatomical structures called gap junctions to facilitate the intercellular cytoplasmic exchange of metabolites, second messengers, and ions.

chapter reviews and discusses recent data supporting a role for hemichannels as pathways for gliotransmission and relevant actors in that tuning of synaptic transmission and plasticity.

2. Structure and major functions of hemichannels

During the past decade, a growing body of evidence began to support a novel mechanism of autocrine/paracrine communication underlying gliotransmission and astrocyte-to-neuron communication: hemichannel-mediated signaling [31]. Each hemichannel is composed of the oligomerization of six protein subunits called connexins around a central pore (**Figure 1**). Connexins embrace a highly conserved protein family encoded by 21 genes in humans and 20 in mice, with orthologs in other vertebrate species [32]. These proteins are abundantly expressed in brain cells [33], including astrocytes [34], and they are named after their predicted molecular mass expressed in kDa, for instance, connexin 43 (Cx43) has a molecular mass of ~43 kDa [35]. For several years, the key function attributed to hemichannels was to constitute the building blocks of the gap junction channels, which are intercellular channels that allow the direct cytoplasmic exchange between contacting cells [35]. Nonetheless, in the 1990s, pioneering findings by Paul and colleagues revealed the presence of functional and solitary hemichannels in "nonjunctional" membranes [36]. Today, it is well accepted that these channels act like aqueous pores, providing a diffusional route of exchange for ions and molecules between the intra- and extracellular space [37]. Across the different tissues, hemichannels allow the cellular release of relevant quantities of autocrine and paracrine signaling molecules (e.g., ATP, glutamate, D-serine, NAD^+, and PGE_2), as well as the influx of other substances (i.e., Ca^{2+} and glucose) [37].

Since their discovery, hemichannels have been linked with cellular damage. This idea came from early studies suggesting that osmotic and ionic imbalances induced by the uncontrolled influx of Na^{2+} and Cl^- through hemichannels could result in further cell swelling and plasma membrane breakdown [36]. In addition, it has been proposed that because hemichannels are permeable to Ca^{2+}, their uncontrolled opening could lead to Ca^{2+} overload and the consequent production of free radicals, lipid peroxidation, and plasma membrane damage [38]. Alternatively, exacerbated hemichannel activity could also induce the release of molecules that at high concentration may be toxic for neighboring cells, such as glutamate, in the case of the CNS [39]. Despite the above, in the last decade, a substantial body of studies has proposed that hemichannels may underpin pivotal neurophysiological functions, such as synaptic efficacy, neural activity, signal processing, cognition, and behavior [27, 28, 40–44].

3. Astroglial hemichannels and their role in synaptic transmission and plasticity

Although rat, mouse, and human astrocytes express abundantly Cx30 and Cx43, as well as Cx26 [45–49], at the moment, Cx43 is the only connexin probed to form functional hemichannels in astrocytes [50]. The opening of astroglial Cx43 hemichannels has been linked with the release of different gliotransmitters (e.g., glutamate, ATP, D-serine, lactate), as well as with the influx of extracellular Ca^{2+} and glucose. Seminal studies by Torres and colleagues demonstrated for the first time that astrocyte hemichannels may act as both sensors and modulators of synaptic

activity [43]. Using UV-photolysis of caged MNI-glutamate in hippocampal slices, they found that specific deletion of Cx43 abrogates ATP-dependent spreading of slow Ca^{2+} waves among astrocytes. Furthermore, these slow Ca^{2+} waves were potentiated when authors used slices from transgenic mice with an astrocyte-targeted point mutation ($Cx43^{G138R}$) that leads to an increased Cx43 hemichannel opening [51]. In addition, they observed that depolarization of inhibitory interneurons from the stratum radiatum reduced CA1 excitatory transmission via the astroglial Cx43 hemichannel-mediated release of ATP and subsequent stimulation of interneuronal P2Y1 receptors [43]. These data shed light for the first time about how astrocyte Cx43 hemichannels may underpin a negative feedback mechanism elicited during sustained excitation to prevent excitotoxicity (**Figure 2**).

Although in normal astrocytes few Cx43 hemichannels are in the plasma membrane and most of them with a low open probability, recent findings have described that they facilitate the release of ATP under basal conditions [27, 41]. Chever and co-workers observed that basal release of ATP via astroglial Cx43 hemichannels is enough to boost the CA1 synaptic transmission triggered by stimulation of Schaffer collaterals, an effect mediated by purinergic receptors [27] (**Figure 2**). Likely the insertion of postsynaptic AMPA receptors as a result of the activation of P2X7 receptors could explain the ATP-dependent potentiation of glutamatergic transmission, as reported before in other brain areas [52]. Astroglial hemichannels also have been found to regulate neuronal activity in the olfactory bulb (OB) [41]. There, the group of Giaume demonstrated that pharmacological inhibition of Cx43 hemichannels decreased the firing and amplitude of depolarized states in mitral cells. Similar findings were observed in mitral cells of OB slices with specific astroglial deletion of Cx43 [41] or in slices treated with A1 adenosine receptor antagonists. These findings denote that likely astrocyte Cx43 hemichannels enhance the amplitude of depolarized states of mitral cells through the release of ATP and its further breakdown to

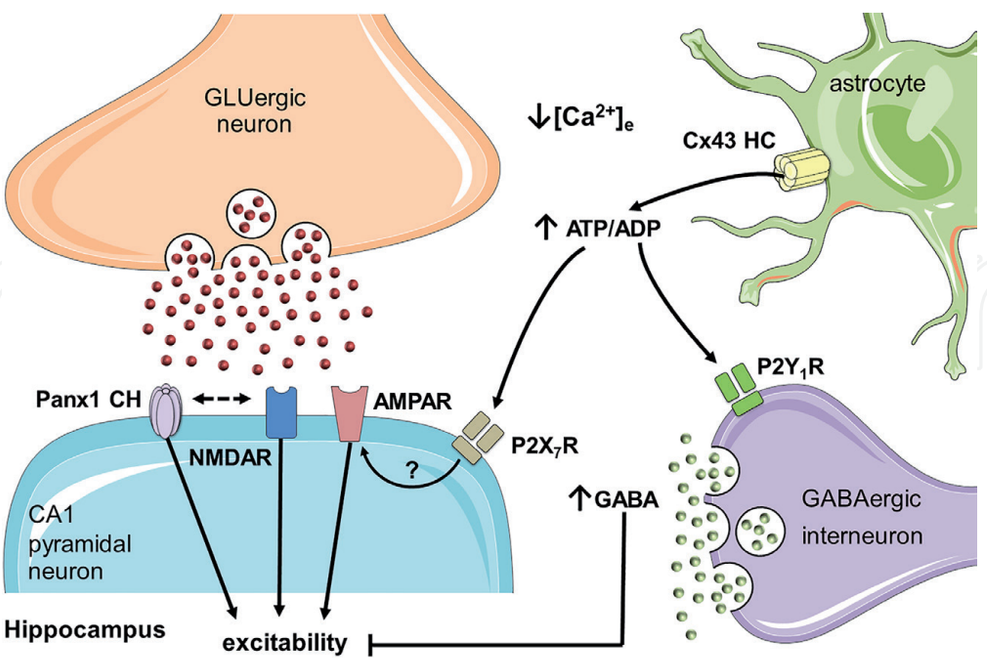

Figure 2.
Possible role of astroglial hemichannels in hippocampal synaptic transmission. During the basal firing of glutamatergic neurons in the hippocampus, Ca^{2+} influx into neurons results in a localized reduction in $[Ca^{2+}]_e$, which in turn opens Cx43 hemichannels (HCs) on astrocytes [43]. The latter lead to the release of ATP, being this crucial for sustaining basal excitatory synaptic transmission [27]. Likely this phenomenon takes place via the activation of P2X7 receptors and further insertion of AMPA receptors in postsynaptic terminals [52]. Alternatively, the conversion of ATP to ADP could depolarize and augment firing in interneurons via P2Y1 receptors, therefore, enhancing inhibitory transmission [43].

adenosine (**Figure 3**). Usually, A1 receptors induce the presynaptic inhibition of glutamate release, reduced postsynaptic NMDAR activation, and decreased Ca^{2+} influx [53]. Therefore, it is possible that the adenosine-mediated enhancement of depolarized states is due to the suppression of inhibitory juxtaglomerular interneurons, as occurred in other brain areas [54] (**Figure 3**).

Astroglial Cx43 hemichannels have been involved not only in synaptic function and transmission but also in synaptic plasticity. High-frequency stimulation (HFS)

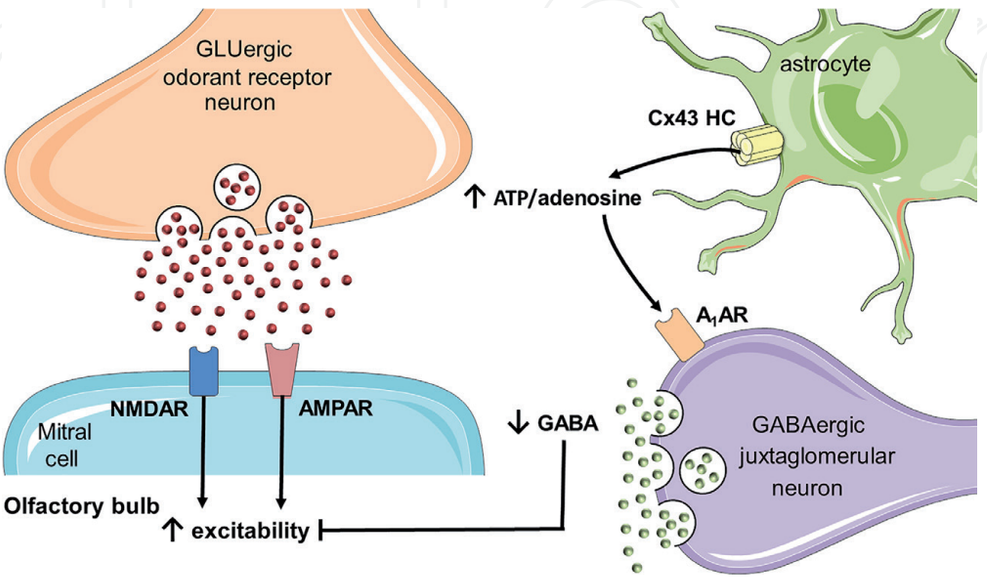

Figure 3.
Implications of astroglial hemichannel activity in neuronal oscillations of the olfactory bulb. Spontaneous neuronal activity in the glomerular layer of the olfactory bulb requires glutamatergic transmission. In this scenario, astrocytes display a basal release of ATP via Cx43 hemichannels (HCs) [41]. The adenosine derived from ATP may reduce the activity of GABAergic inhibitory juxtaglomerular neurons through the stimulation of A1 adenosine receptors. This permits the basal slow oscillations of up and down states of mitral cells in the olfactory bulb.

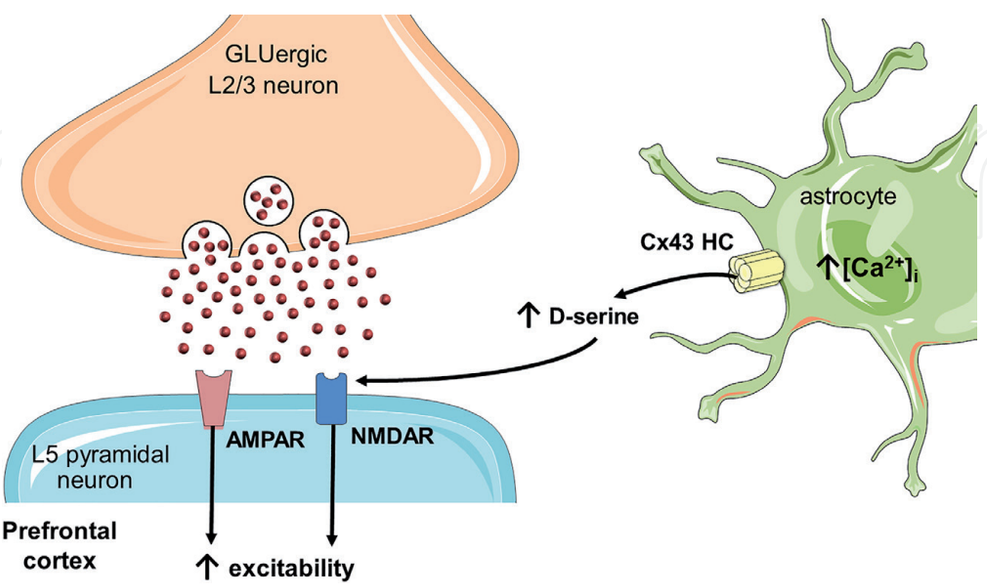

Figure 4.
Astroglial hemichannels and their impact on synaptic plasticity in the prefrontal cortex. In the prefrontal cortex, continuous stimulation of layer 2/3 neurons induces long-term potentiation (LTP) of NMDA and AMPA receptor currents in layer 5 pyramidal neurons. In this context, $[Ca^{2+}]_i$ is needed for the opening of Cx43 hemichannels (HCs) in astrocytes [28], which cause release of D-serine. This gliotransmitter facilitates LTP of NMDA and AMPA excitatory synaptic currents mediated by high-frequency stimulation.

of neuronal layer 2/3 (L2/3) triggers glutamatergic synaptic transmission in pyramidal cells at layer 5 (L5) of the prefrontal cortex (PFC) [55]. In this context and using PFC slices, Meunier and colleagues observed that genetic ablation of Cx43 or inhibition of Cx43 hemichannels strongly counteracts the NMDAR-dependent excitatory postsynaptic currents (EPSCs) and increases AMPA/NMDA current ratio induced by HSF in L5 [28]. Relevantly, the latter responses did not occur when D-serine was added at the recording media, revealing that the release of D-serine and astroglial hemichannel function are linked and modulate NMDAR-dependent synaptic transmission in PFC pyramidal cells. Furthermore, when $[Ca^{2+}]_i$ was clamped or D-serine production was inhibited in the L5 astroglial network, HFS failed to potentiate the NMDAR-dependent synaptic currents [28] (**Figure 4**). Accordingly, the authors hypothesized that potentiation of glutamatergic transmission at the PFC relies on $[Ca^{2+}]_i$-mediated opening of astroglial Cx43 hemichannels and the further release of D-serine (**Figure 4**).

The impact of astroglial hemichannels on synaptic transmission and plasticity has a subsequent echo on higher brain function and behavior. Indeed, in vivo inhibition of Cx43 hemichannels at the basolateral amygdala causes transitory and specific amnesia for auditory fear conditioning [42]. Remarkably, learning capacity was restored by the co-administration of a cocktail of supposed gliotransmitters (lactate, glutamate, D-serine, glutamine, glycine, and ATP), evidencing for the first time a physiological involvement for astroglial Cx43 hemichannels in higher brain function. In the same line, a recent study found that intraventricular administration of Gap19, a specific Cx43 hemichannel blocker [56], significantly impairs the spatial short-term memory, as assayed with the delayed spontaneous alternation Y maze task [44].

4. Conclusions

The impact of functional astroglial hemichannels in synaptic transmission and plasticity may depend on the number of channels available in the plasma membrane, their open probability, and their conductance and/or selectivity. Of particular relevance is to disentangle how synaptic function is modulated by regulations in gating properties of astroglial hemichannels, as well as changes in their trafficking or de novo synthesis. Elucidating the latter will allow us to understand whether hemichannel opening in astrocytes tunes the temporal outcome for sculpting either short-term (milliseconds to a few minutes) or long-term (minutes to hours) plasticity in the nervous system. One point of concern is the urgent need of developing new molecular and pharmacological tools to specifically dissect the contribution of astroglial hemichannels to the function of neural networks without affecting other hemichannel-forming proteins in other brain cells (e.g., microglia, oligodendrocytes, and endothelial cells). Finally, although growing evidence in ex vivo preparations has extended our knowledge about the role of astroglial hemichannels in gliotransmission, additional data are necessary to demonstrate whether this truly occurs in vivo.

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Conflict of interest

The author declares no conflict of interest.

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