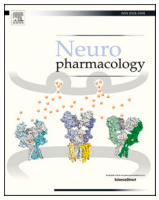
Neuropharmacology 231 (2023) 109509

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Receptor-receptor interactions and microvesicle exchange as mechanisms modulating signaling between neurons and astrocytes

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ARTICLE INFO

Handling Editor: Bruno Frenguelli

Keywords:
Tripartite synapse
Astrocytes
Receptor-receptor interactions
Oligomerization
Exosomes
Penta-partite synapse

ABSTRACT

It is well known that astrocytes play a significant metabolic role in the nervous tissue, maintaining the homeostasis of the extracellular space and of the blood-brain barrier, and providing trophic support to neurons. In addition, however, evidence exists indicating astrocytes as important elements for brain activity through signaling exchange with neurons. Astrocytes, indeed, can sense synaptic activity and their molecular machinery responds to neurotransmitters released by neurons with cytoplasmic Ca^{2+} elevations that, in turn, stimulate the release of neuroactive substances (gliotransmitters) influencing nearby neurons. In both cell types the recognition and transduction of this complex pattern of signals is mediated by specific receptors that are also involved in mechanisms tuning the intercellular cross-talk between astrocytes and neurons. Two of these mechanisms are the focus of the present discussion. The first concerns direct receptor-receptor interactions leading to the formation at the cell membrane of multimeric receptor complexes. The cooperativity that emerges in the actions of orthosteric and allosteric ligands of the monomers forming the assembly provides the cell decoding apparatus with sophisticated and flexible dynamics in terms of recognition and signal transduction pathways. A further mechanism of plasticity involving receptors is based on the transfer of elements of the cellular signaling apparatus via extracellular microvesicles acting as protective containers, which can lead to transient changes in the transmitting/decoding capabilities of the target cell.

This article is part of the Special Issue on "The receptor-receptor interaction as a new target for therapy".

1. Introduction

In the nervous tissue the position of astrocytes allows them to act as a conduit for the routing of signals between different cell types. Astrocytes, indeed, are polarized into two functional domains: the largest portion of the astrocyte membrane makes contact with neurons, and the remainder abut the capillaries, forming the astrocytic end-foot processes. Astrocytes, therefore, have the potential to shuffle nutrients and metabolites between the blood supply and the neurons (Araque et al., 2001). This structural association led to the initial views about astrocytes as cells basically involved in metabolic roles within the nervous system and a number of astrocyte homeostatic functions supporting neuronal life have been identified. They include the induction and maintenance of the blood-brain barrier, the uptake of nutrients from the capillaries (Benarroch, 2005) and the trophic support to neurons (Suzuki et al., 2011), the regulation of the neuronal microenvironment in terms of water, pH, ions (e.g. K^+) and extra-cellular matrix molecules concentration (MacAulay et al., 2004; Hubbard et al., 2018).

However, since single astrocytes can make contacts with multiple neurons, these non-neuronal cells are also positioned to provide information transfer between neighboring neurons. In particular, ultrastructural investigations (Ventura and Harris, 1999) indicated that an overwhelming number of thin filopodia- and lamellipodia-like process terminals (called PAP and accounting for 70%–80% of the astrocytic plasma membrane) contact and enwrap synapses, the sites of neuronal communication, sometimes completely encapsulating them. Even if PAP are found in all brain regions, the proportion of synapses having them and the level of synaptic coverage vary significantly and it should be underlined that enwrapping of synapses by PAP is an important feature that allows high efficiency and privacy of the transmission. Thus, the function and efficacy of synaptic transmission are determined not only

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https://doi.org/10.1016/j.neuropharm.2023.109509
Received 17 December 2022; Received in revised form 21 February 2023; Accepted 16 March 2023
Available online 17 March 2023

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CNS	central nervous system
ECS	extracellular space
EMV	extracellular microvesicles
GABA	γ-aminobutyric acid
GPCR	G protein-coupled receptor
mGluR	metabotropic glutamate receptor
NMDA	*N*-methyl-D-aspartate
PAP	perisynaptic astrocyte processes
RRI	receptor-receptor interactions
SPARC	secreted protein acidic and cysteine rich
TfR2	transferrin receptor 2
VT	volume transmission

by the composition and activity of pre- and postsynaptic components but also by the features of the PAP that enwrap the synapse. This evidence led to the proposal of the concept of "tripartite synapse" (Araque et al., 1999). According to this view, the relationship between astrocytes and neurons is a bidirectional one, with synaptic activity influencing astrocytic activation, which in turn modulates the activity of neurons (Sancho et al., 2021). In fact, as demonstrated in the late 1980s and early 1990s (see (Araque et al., 2001)), neurotransmitters elevate astrocytic calcium levels as the result of the release of calcium from internal stores. By exploiting gap junctions between the cells (Allen and Barres, 2005) this wave of intracellular calcium elevation as been shown to propagate between astrocytes for hundreds of micrometers (Cornell et al., 1990), leading to the release of gliotransmitters (such as D-serine, ATP, glutamate) from astrocytes and to a direct regulation of ongoing synaptic activity (Fellin and Carmignoto, 2004).

The intercellular communication between neurons and astrocytes and between astrocytes, however, is not limited to specific districts of the cells, such as synaptic regions or gap junctions (where the involved cells are in contiguity), but it also includes processes of VT, based on the release of signaling molecules and their diffusion in the ECS (see (Guidolin et al., 2017) for a review) to reach distant targets. In neurons such a diffuse, non-synaptic, mode of intercellular communication was identified by studies performed in the late 1970s (see (Vizi, 1980; Agnati et al., 1986)), while the concept of astrocytes as secretory cells is almost as old as the discovery of these glial cells (von Lenhossék, 1895), being the release and uptake of substances the main mode exploited by astrocytes to perform their metabolic roles in the CNS (Vardjan and Zorec, 2015; Verkhratsky et al., 2016).

In this respect, however, a functionally important aspect to consider is the motile nature of PAP that have the ability to rapidly restructure their thin-branched processes modifying their coverage of the synaptic elements (Bernardinelli et al., 2014). Several studies, indeed, describe them as plastic structures able to change their morphology within minutes, thus modifying their coverage of pre- and postsynaptic elements (see (Reichenbach et al., 2010; Bernardinelli et al., 2014)). In view of these data, it has been suggested (Marcoli et al., 2015) that a sophisticated control of the PAP's plasticity could allow moving from a high privacy of the synaptic transmission (close enwrap of the synapse) to a more or less broad opening of the enwrapping. This would lead to diffusion by VT of signaling molecules (such as neurotransmitters, neurotransmitter precursors and neuromodulators) to neighboring astrocytes, neurons and other glial cells and to a modulation of their activity (Grillner and Graybel, 2006). To account for this more complex network of signaling, more recently the concept of "penta-partite synapse", whose dynamics involves not only neuronal synapses and astrocytes but also the extracellular matrix and microglial cells, has been proposed (Agnati et al., 2018; Aramideh et al., 2021).

It is well known that at the cellular level specific receptors are involved in the recognition and transduction of this pattern of signals and, notably, astrocytes can express many neurotransmitter receptors also expressed by neurons (see (Nedergaard and Verkhratsky, 2012)). In the last decades several *in vitro* and *in vivo* experiments demonstrated that receptors may establish structural, allosteric, receptor-receptor interactions (RRI) (Agnati et al., 1983; Fuxe et al., 1983). The term RRI refers to an interaction requiring a direct physical contact between the involved receptor proteins leading to the formation at the cell membrane of multimeric receptor complexes (see (Guidolin et al., 2019) for a recent review). This evidence indicated that oligomeric organization represents a quite common feature in the different receptor families, with the ion channel receptors (where multimerization is needed) lying at one end of the spectrum and GPCRs at the other. Thus, as pointed out by Changeux and Christopoulos in a detailed review (Changeux and Christopoulos, 2017), oligomerization emerges as an efficient mechanism for tuning the functionality of receptor proteins (including those able to signal as monomers, such as GPCRs) leading to a sophisticated regulation of the intercellular communication already at the plasma membrane level (Guidolin et al., 2017).

A further mechanism of plasticity involving receptors has been reported in several cell types (Simons and Raposo, 2009). It is based on a specific form of VT, which involves the transfer of elements of the cellular signaling apparatus via EMV acting as protective containers and can lead to transient changes in the transmitting/decoding capabilities of the target cell (Agnati et al., 2014). Available data concerning these modulatory processes will be the focus of the present discussion, with special reference to the role they can play in the neuron-astrocyte cross-talk.

2. Receptor complexes and modulation of intercellular signaling

At the molecular level, a key mechanism regulating intercellular communication (and, in neurons, synaptic efficiency) is receptor trafficking composed of lateral (between different cell districts) and vertical (between intracellular stores and cell membrane) mobility of receptors (see (Vizi et al., 2010) for a review). In addition, the existence of RRI can lead to the assembly of oligomeric receptor complexes with different properties even if formed by the same types of monomers (Agnati et al., 2010a; Guidolin et al., 2019). The basic biochemical mechanism leading to the formation of these receptor assemblies are allosteric interactions, and, as outlined by Changeux and Christopoulos (Changeux and Christopoulos, 2017), the cooperativity that emerges in the actions of orthosteric and allosteric ligands of the monomers forming the assembly provides the cell decoding apparatus with sophisticated and flexible dynamics (see (Farran, 2017; Guidolin et al., 2018) for specific reviews) in terms of recognition and signal transduction pathways. When protomers, indeed, establish direct RRI leading to a quaternary structure, energy perturbations occurring at some site of one protomer can propagate over the interface between receptors into the nearby protomers, changing their conformational and functional properties, strongly influencing the chain of events linking ligand recognition to signal transduction from the single protomers. A further relevant aspect of receptor complex formation is the possibility that novel specific allosteric sites suitable for the binding of some modulators could appear in the quaternary structure resulting from the assemblage of protomers (Agnati et al., 2008). Thus, ligands specific to the receptor complex as such may also exist.

In this context, the sections that follow will be specifically focused on receptors and receptors complexes mediating the bidirectional neuron-astrocyte signaling at the tripartite/penta-partite synapse.

2.1. Receptors involved in astrocyte to neuron communication

Astrocytes can release a large number of neuroactive substances controlling different aspects of neuronal development and function (see

(Araque et al., 2001; Kofuji and Araque, 2021)). In the context of the present discussion of particular interest are the regulatory effects on synaptic transmission and plasticity of some gliotransmitters that will be here briefly addressed.

Glutamate was the first gliotransmitter identified as mediator of astrocyte to neuron signaling (Araque et al., 1998a; Angulo et al., 2004). Astrocytic glutamate was reported to trigger neural calcium elevation by acting on NMDA receptors (Araque et al., 1998b, 2001) and to regulate synaptic efficacy by activating mGluRs (Andersson et al., 2007; Gomez-Gonzalo et al., 2017; Covelo and Araque, 2018; Kofuji and Araque, 2021). In the hippocampus, for instance, the activation of type 1 mGluRs by astrocyte glutamate induces a short-term enhancement of synaptic efficacy (Gomez-Gonzalo et al., 2017; Covelo and Araque, 2018), while a heterosynaptic depression has been found to be mediated by type 2/3 mGluRs activated by astrocyte glutamate (Andersson et al., 2007).

NMDA receptor signaling is also modulated by another gliotransmitter released by astrocytes, namely D-serine, acting as co-agonist of these receptors. D-serine, indeed, was shown to be an endogenous ligand of synaptic NMDA receptors (Papouin et al., 2012), just as glycine is a co-agonist of extra synaptic NMDA receptors, and was shown to be responsible for modulating NMDA receptor-mediated synaptic transmission in cultured hippocampal neurons (Mothet et al., 2000). In this respect, it is of interest to observe that synaptic NMDA receptors, but not extrasynaptic NMDA receptors, mediate long-term potentiation, an important form of synaptic plasticity and silencing synaptic NMDA receptors is also protective against NMDA-induced exitotoxicity (Papouin et al., 2012).

Purinergic signaling mediated by activation of neuronal GPCRs can also lead to synaptic regulation in different brain areas through several mechanisms. ATP released from astrocytes can be metabolized by extracellular ATPases to produce adenosine, the endogenous ligand of A_1 and A_{2A} receptors (Kofuji and Araque, 2021). Furthermore, in experimental models of hypoxia a direct astrocytic release of adenosine has been shown to occur (Martin et al., 2007). Interestingly, the release of ATP/adenosine from the same astrocyte can differentially affect specific neuronal circuits depending on the presynaptic receptors expressed. In the centromedial nucleus of the amygdala, for instance, astrocytic ATP/adenosine was shown to depress excitatory neurotransmission from the basolateral amygdala by activating presynaptic A_1 receptors, whereas it enhanced synaptic transmission of inhibitory inputs from the central lateral amygdala through presynaptic A_{2A} receptors (Martin-Fernandez et al., 2017). Remarkably, data were also provided showing that following a prolonged increase of adenosine concentration (as occurs during ischemia) which leads to A_{2A} receptors activation, A_1 receptors signaling and the neuroprotective A_1-mediated effects were significantly reduced (Pedata et al., 2001; Franco et al., 2021).

GABA is the main inhibitory transmitter in the central nervous system and the extracellular levels of GABA are controlled by the activity of GABA transporters in astrocytes (Schousboe et al., 2017). In addition, astrocytes are able to release GABA (Yoon and Lee, 2014; Gaidin et al., 2020) which acting on neuronal $GABA_B$ receptors may provide synaptic regulation in different brain areas. Examples include the cerebellum, where astrocytic GABA regulates the synapses between parallel fiber and Purkinje cells contributing to motor coordination (Woo et al., 2018), and the hippocampus, where a disinhibition of excitatory synapses from the perforant path to dentate granule neurons is provided by astrocytic GABA acting on the $GABA_B$ receptors of GABAergic interneurons (Yarishkin et al., 2015).

2.2. Receptors involved in neuron to astrocyte communication

Glutamate is the main excitatory neurotransmitter in the CNS and acts on astrocytes as well. Glutamatergic signaling to these cells is mainly mediated by mGlurRs, being $mGluR_1$, $mGluR_3$ and $mGluR_5$ the glutamate receptors mainly expressed by astrocytes. In this context, however, a quite large evidence indicates type 5 mGluR as the most relevant glutamate receptor in astrocytes (Kofuji and Araque, 2021), mediating astrocytic responses to glutamate in many brain areas such as hippocampus, nucleus accumbens and thalamus.

For what it concerns GABA, both ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors are expressed by astrocytes (Fraser et al., 1994; Charles et al., 2003). The activation of $GABA_B$ triggers Ca^{2+} events in astrocytes that were shown to involve G proteins and Ca^{2+} release from internal stores (Meier et al., 2008), and that can be blocked by the $GABA_B$ receptor antagonist CGP55845A (Kang et al., 1998; Perea et al., 2016). Intriguing are findings indicating that activation of $GABA_B$ receptor signaling in astrocytes can potentiate glutamatergic transmission of pyramidal neurons (Perea et al., 2016). Thus, an inhibitory GABA signal has the potential to become an excitatory signal through astrocyte activation for selective neuron populations in peculiar brain regions.

Purinergic signaling involving ATP or adenosine as neurotransmitters was found to operate at many synapses in the CNS (Abbracchio et al., 2009). In astrocytes a number of adenosine receptors subtypes has been identified (A_1 and A_{2A} receptors being the most represented (Boison et al., 2010)) and both ionotropic P2X and metabotropic P2Y ATP receptors were reported to be expressed (Abbracchio and Ceruti, 2006). The presence of multiple purinergic receptors at the same astrocyte may lead to an abundance of effects, a quite common feature being an increase in intracellular Ca^{2+} (see (Kofuji and Araque, 2021) for a recent discussion of the topic). In this respect, however, it has to be observed that while the activation of A_{2A} receptor, being coupled to G_s protein, positively affect a Ca^{2+} response, activation of A_1 receptor (coupled to G_i protein) do not influence calcium levels (Alloisio et al., 2004).

A number of other neurotransmitters, including dopamine, noradrenaline, acetylcholine and cannabinoids, may also act on astrocytes. Dopamine D_2 receptor activation was reported to decrease intracellular Ca^{2+} levels in hippocampal (Jennings et al., 2017) and ventral midbrain astrocytes (Xin et al., 2019), while D_1 receptor activation elevated astrocytic Ca^{2+} levels in hippocampus (Jennings et al., 2017) and nucleus accumbens (Corkrum et al., 2020). Astrocytes also express α_1, α_2 and β_1 adrenergic receptors (Hertz et al., 2010) allowing them to respond to neuronal noradrenaline (Ding et al., 2013). Muscarinic and nicotinic acetylcholine receptors have been identified in astrocytes (Guizzetti et al., 2008; Hernandez et al., 2014) where they mediate processes of neuron to astrocyte communication involved in different forms of synaptic plasticity (see (Kofuji and Araque, 2021)), and several forms of synaptic plasticity have also been linked with cannabinoid signaling to astrocytes (Navarrete and Araque, 2010), that express CB_1 cannabinoid receptors, although at lower levels as compared to neurons (Navarrete et al., 2014).

2.3. Receptor complexes and neuron-astrocyte cross-talk

A large pool of experimental evidence (see www.gpcr-hetnet.com (Borroto-Escuela et al., 2014) for what it concerns GPCRs) indicates that almost all of the abovementioned receptors involved in neuron-astrocyte cross-talk are able to be part of receptor complexes. Examples of experimentally identified heteromers in neurons and astrocytes are reported in Table 1. As shown, many of these complexes (e.g. $mGluR_5$-A_{2A}, NMDA-D_2, $GABA_B$-M_2, A_{2A}-D_2, A_1-A_{2A}, CB_1-D_2) are formed by receptor protomers recognizing different signals directly involved in neuron-astrocyte communication, suggesting receptor complexes as a relevant molecular organization to tune and integrate the intercellular cross-talk between astrocytes and neurons. Glutamate receptor subtypes, in particular, appear to participate in the formation of a large number of heteromers located in relation to glutamate synapses, especially in the basal ganglia (Borroto-Escuela et al., 2018), a finding of interest in view of the key role of the bidirectional interaction between neurons and astrocytes at the central excitatory synapses (Benarroch, 2005). In addition, some heteroreceptor complexes (like $mGluR_5$-MOR,

Table 1
Receptor complexes containing receptors involved in neuron-astrocyte cross-talk.

Receptor complex	Location	Reference
$mGluR_1$-A_1	Neurons	Ciruela et al. (2001)
$mGluR_1$-$mGluR_5$	Neurons	Goudet et al. (2005)
$mGluR_3$-$mGluR_5$ (putative)	Astrocytes	Di Menna et al. (2017)
$mGluR_5$-A_{2A}	Neurons	Borroto-Escuela et al., 2017a
$mGluR_5$-D_2	Neurons	Cabello et al. (2009)
$mGluR_5$-MOR	Neurons	Schroder et al. (2009)
NMDA-$mGluR_5$	Neurons	Perroy et al. (2008)
NMDA-D_1	Neurons	Lee et al. (2002)
NMDA-D_2	Neurons	Liu et al. (2006)
NMDA-A_{2A}	Neurons	Franco et al. (2020); Tan et al. (2021)
NMDA-MOR	Neurons	Rodriguez-Munoz et al. (2012)
NMDA-CB_2	Neurons	Rivas-Santisteban et al. (2021)
NMDA-H_3	Neurons	Rodriguez-Rui et al. (2017)
$GABA_B$-$mGluR_1$ (putative)	Neurons	Hirono et al. (2001)
$GABA_B$-M_2	Neurons	Boyer et al. (2009)
$GABA_B$-$SSTR_4$ (probable)	Astrocytes	Mariotti et al. (2018)
A_{2A}-D_2	Neurons	Trifilieff et al. (2011)
	Astrocytes	Pelassa et al. (2019)
A_{2A}-OTR	Astrocytes	Amato et al., 2022
A_1-A_{2A}	Neurons	Ferré et al. (2008)
	Astrocytes	Franco et al. (2021)
A_1-$P2Y_1$	Astrocytes	Tonazzini et al. (2008)
D_1-D_2	Neurons	Rashid et al. (2007)
D_2-$5HT_1$	Astrocytes	Kolasa et al. (2018)
D_2-GHS_{1A}	Neurons	Kern et al. (2012)
CB_1-D_2	Neurons	Przybyla and Watts (2010)
CB_1-CB_2	Neurons	Callén et al. (2012)
A_{2A}-D_2-$mGluR_5$	Neurons	Beggiato et al., 2016
A_{2A}-D_2-CB_1	Neurons	Carriba et al. (2008); Pinna et al. (2014)

MOR, μ-opioid receptor; H_3, type 3 histamine receptor; M_2, type 2 muscarinic receptor.
OTR, oxytocine receptor; GHS_{1A}, type 1a ghrelin receptor; $SSTR_4$, type 4 somatostatin receptor.

A_{2A}-OTR, D_2-GHS_{1A}) seem to indicate that the neuron-astrocyte interaction could also be regulated by other lines of signaling from the neuronal environment not directly involved in the intercellular communication between neurons and astrocytes.

In the last decades the integrative mechanisms provided by heteroreceptor complexes to regulate intercellular communication have been the target of intensive research efforts and specific reviews on the topic are presently available (see (Ferré et al., 2014; Fuxe and Borroto-Escuela, 2016; Farran, 2017; Guidolin et al., 2018, 2019)).

In the context of the present discussion, the A_{2A}-D_2 heteromer, expressed both in neurons and astrocytes, may constitute a representative example (see (Guidolin et al., 2020)). Both adenosine A_{2A} and dopamine D_2 receptors are class A (rhodopsin-like) GPCRs, characterized by the typical seven transmembrane α-helix structure (see Fig. 1). A_{2A} receptor couples primarily to members of the G_s family of G proteins and signaling involves activation of adenylate cyclase and generation of cAMP (see Fredholm et al., 2007). The A_{2A} receptor can also be the source of a type of signaling not directly related to G proteins, but based on the recruitment of β-arrestin (Khoa et al., 2006; Fredholm et al., 2007). In the brain A_{2A} receptor plays important roles in the regulation of glutamate and dopamine release and is also of importance for the time course of caffeine action and for that of other drugs as well (see Ferré et al., 2007; Fredholm et al., 2007). Dopamine D_2 receptor primarly couples to the G protein $G_{i/0}$ whose activation inhibits adenylate cyclase activity (see Zhuang et al., 2021). In the brain, aberrant D_2 signaling has been associated with many neuropsychiatric diseases, including Parkinson's disease, schizophrenia, autism, attention-deficit hyperactivity disorder, drug abuse and various types of cognitive impairement (Zhuang et al., 2021). Both in experimental animals and in humans, adenosine A_{2A} receptors and dopamine D_2 receptors were both found highly concentrated in the striatum, the main input structure of the basal ganglia (Ferré et al., 1997; Schiffmann et al., 2007). Concerning neurons, A_{2A} and D_2 receptors resulted predominantly expressed by the GABAergic enkephalinergic neurons (Ferré et al., 1997; Agnati et al., 2003; Schiffmann et al., 2007) and the A_{2A}-D_2 receptor-receptor interaction leading to the formation of heteromers was demonstrated in both the somatodendritic area and in the nerve terminals of these neurons (Fuxe et al., 1998, 2003). In the striatum, however, evidence exists indicating their co-localization also in the striatal cholinergic interneurons (Tozzi et al., 2011) and in the cortico-striatal glutamate terminals (Fuxe et al., 2007), where they modulate glutamatergic transmission (Tozzi et al., 2007). From the pharmacological standpoint, experimental evidence showed that the receptor complex formation

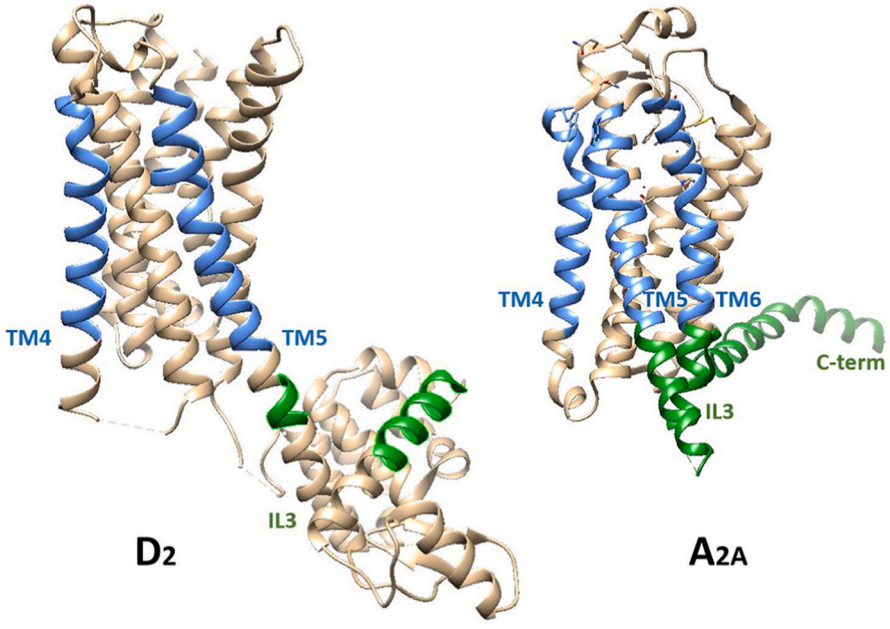

Fig. 1. Possible interaction interfaces on dopamine D_2 (PDB code: 6CM4) and adenosine A_{2A} (PDB code: 2YDO) receptors, as predicted by bioinformatics analyses (see (Guidolin et al., 2019)). The identified domains are indicated.

modifies the signaling from the single protomers. In particular, antagonistic interactions occur in the A_{2A}-D_2 heterodimer, as demonstrated by early *in vitro* experiments in membrane preparations after incubation with the A_{2A} agonist CGS21680 leading to a reduction of the affinity of the high affinity D_2 agonist-binding site (Ferré et al., 1991; Fuxe et al., 1998). This finding was subsequently confirmed in brain tissue by receptor autoradiography studies, showing a strong reduction in D_2 receptor affinity for dopamine after A_{2A} receptor agonist treatment in the nucleus accumbens core and shell of rats and humans (Diaz-Cabiale et al., 2001). A reciprocal antagonistic interaction between D_2 and A_{2A} receptors also exists. D_2 receptor activation, indeed, was found to inhibit the A_{2A}-induced increase in cAMP accumulation via $G_{i/o}$ at the level of the adenylate cyclase (Kull et al., 1999) and was shown to slow and partially inhibit the binding association of a fluorescent A_{2A} agonist to its receptor (Fernandez-Dueñas et al., 2012). Antagonistic interactions between A_{2A} and D_2 receptors were also recently demonstrated in astrocytes by using biochemical and biophysical techniques, such as co-immunoprecipitation and proximity ligation assay (Cervetto et al., 2016; Pelassa et al., 2019). A_{2A} and D_2 receptors were found co-expressed on the same astrocyte processes where they can form receptor heteromers. From the functional standpoint, the effect of A_{2A}-D_2 RRI was analyzed by measuring *in vitro* the release of the gliotransmitter glutamate following the administration of the D_2 receptor agonist quinpirole and the A_{2A} receptor agonist CGS21680 (Cervetto et al., 2016). The activation of D_2 receptors inhibited glutamate release, while the activation of A_{2A} receptors, per se ineffective, abolished the release inhibition induced by D_2 activation. Interestingly, the administration of the synthetic peptide VLRRRRKRVN, which can interfere with the D_2 receptor domain involved in electrostatic interactions critical to receptor heteromerization (Woods et al., 2005), eliminated the A_{2A}-mediated inhibition of the response to D_2 receptor activation, confirming that receptor complexes were responsible for the observed effect.

The A_{2A}-D_2 heteromer also provides an example of a second modulatory mechanism associated with receptor complex formation, namely the possible formation of new allosteric sites specific to the complex allowing for the binding of some ligand. In the A_{2A}-D_2 complex, indeed, homocysteine can bind to the heterodimer without interfering with the RRI between A_{2A} and D_2, but acting as an allosteric antagonist of the D_2 receptor (Agnati et al., 2008). Thus, it also amplifies the inhibitory effect of A_{2A} agonists. Homocysteine-induced modulatory actions were demonstrated in striatal neurons (Agnati et al., 2006, 2008), as well as in astrocytes (Cervetto et al., 2018) where it reduces the D_2-mediated inhibition of glutamate release.

A feature of receptor proteins (GPCRs in particular) predicted by a variety of bioinformatics methods (see (Guidolin et al., 2011)) is illustrated in Fig. 1 where A_{2A} and D_2 receptors are used as examples. Receptor proteins, indeed, in general exhibit multiple possible interaction interfaces opening the possibility for the formation of oligomers with more than two protomers. Actually, the numbers of ways receptors interact in the membrane to form complexes is probably limited, the vast majority of experimentally identified receptor complexes being dimers. Furthermore, some interfaces have been observed to be more exploited than others for RRI (Borroto-Escuela et al., 2018). Nevertheless, trimeric heteroreceptors have been detected. Interesting examples are the A_{2A}-D_2-CB_1 (Carriba et al., 2008; Pinna et al., 2014) and the A_{2A}-D_2-$mGluR_5$ (Cabello et al., 2009; Beggiato et al., 2016; Borroto-Escuela et al., 2018) heteromers. In the brain, A_{2A}-D_2-CB_1 heterotrimer has been identified by radioligand binding protocols in the striatum (Pinna et al., 2014), while using high-resolution immunoelectron microscopy (Cabello et al., 2009) A_{2A}-D_2-$mGluR_5$ heterotrimer was suggested to exist extrasynaptically around striatal glutamate synapses. This first direct anatomic evidence for $mGluR_5$, D_2 and A_{2A} receptors' codistribution in the same neuronal compartment, together with evidence on the existence of strong multiple functional interactions among the three receptors, supported the notion that these receptors form a complex in GABAergic striatopallidal neurons (Beggiato et al., 2016). The stoichiometry and the spatial organization of this heteroreceptor complex is still unknown. However, based on the behavioral findings and the modulation of D_2 binding obtained upon coactivation of A_{2A} and $mGluR_5$, the allosteric RRI in this heterocomplexes lead to an effective inhibition of D_2 recognition and signaling (Popoli et al., 2001; Beggiato et al., 2016). Thus, in view of their composition in terms of protomers both these trimeric heteroreceptor complexes may represent a molecular organization significantly modulating astrocyte to neuron communication.

3. Intercellular transfer of recognition/decoding apparatuses

In the last decade, evidence was obtained that cells can exchange a set of chemical messages via EMV acting as protective containers (Février and Raposo, 2004; Simons and Raposo, 2009). They can transport Rab GTPases, tetraspanins, cholesterol, sphingolipids, ceramide and receptor proteins. Within them there are also subsets of mRNAs and non-coding regulatory micro RNAs (miRNA) (see (Borroto-Escuela et al., 2015)). This class of intercellular communication, therefore, belongs to the VT mode of communication (virtually no continuous channel). This peculiar class of VT has also been called 'roamer type of VT' (Agnati et al., 2010b, 2014), based on an analogy between vesicles and itinerant workers who move away from the source of production and roam from cell to cell with their set of products.

Different types of EMV have been described (see (Lakkaraju and Rodriguez-Boulan, 2008; Agnati et al., 2014)) but two types are of major importance for our discussion. Exosomes are vesicles (40–100 nm in diameter) contained in the so-called early, late or recycling endosomes, a type of multivesicular bodies (Lakkaraju and Rodriguez-Boulan, 2008). Endosomes usually transport newly synthesized material from the Golgi complex. Extracellular vesicles can also be formed from lipid raft domains of the plasma membrane and are then called shedding vesicles (Smalheiser, 2007). Thus, shedding vesicles show surface markers that are dependent on the composition of the membrane of origin and constitute a larger and more heterogeneous population of extracellular vesicles.

Studies on cultured cortical nerve cells demonstrate that EMV can be released from nerve cells (Fauré et al., 2006), and exosomes were proposed to be a new way of interneuronal communication (Chivet et al., 2013; Agnati et al., 2014; Guidolin et al., 2017). In the CNS, indeed, transfer of chemicals among nerve cells may take place likely involving mainly release of EMV from soma, which have a significant capacity to release exosomes in view of their origin in endosomes-multivesicular bodies (Borroto-Escuela et al., 2015). EMV-mediated intercellular exchanges between nerve cells appear to play myriad roles in CNS homeostasis (see (Lizarraga-Valderrama and Sheridan, 2021) for a recent extended review on this topic). Of particular interest for the present discussion are experimental findings showing that a number of elements of the cell signaling/decoding apparatus (or regulatory materials modifying it) can be transferred from one to another cell type via the exosome pathway (see (Guidolin et al., 2017) for details), leading the target cell to transiently acquire a new phenotype, that enables it to recognize/decode signals for which the cell does not express the pertinent cognate receptors.

In this respect, the section that follows will briefly address available data on this topic, with special reference to the signaling between neurons and astrocytes. They are also summarized in Table 2.

3.1. Microvesicle exchange between neurons and astrocytes modulating their decoding apparatus

EMV released from neurons were found to modulate synaptic plasticity (Lizarraga-Valderrama and Sheridan, 2021). As shown by Goldie and collaborators (Goldie et al., 2014), indeed, depolarization of human neuroblasts (SH-Sy 5Y cells) was associated to a decrease of miRNA expression in the neurites and to a release of endosomes enriched with a

Table 2
Transfer by EMV of materials modulating the cell signaling/decoding apparatus.

EMV type	Source	Cargo	Reference
Exosome	Glutamatergic neurons	AMPA receptors	Lachenal et al. (2011)
Exosome	Cortical neurons	AMPA receptors	Fauré et al. (2006)
Exosome	Neurons	miR-124a	Morel et al. (2013)
Exosome	Glioblastoma U87MG cells	A_{2A}, D_2 receptors	Guescini et al. (2012)
Exosome	Astrocytes	miR-223	Amoah et al. (2020)
Exosome	Astrocytes	SPARC protein	Vincent et al. (2008)
Exosome	Astrocytes	$IL1R_2$, CCR_2, $CXCR_4$ receptors (putative)	Fuxe et al. (2013)

Legends.

subset of these molecules and with the microtubule-associated protein 1B (MAP1B). Four of these miRNA (miR-638, miR-149*, miR-4281 and let-7e) may regulate in the recipient cells the expression and translation of functional mRNA involved in plasticity-related processes, including synaptic activity, protein localization and morphogenesis (Goldie et al., 2014). MAP1B, on the other side, is known to play key roles in axon guidance, neurite branching and in the regulation of postsynaptic spines morphology (Barnat et al., 2016). In this context, of particular interest is evidence (Fauré et al., 2006; Lachenal et al., 2011) indicating that glutamatergic neurons release AMPA receptors-containing exosomes, suggesting a mechanism to decrease AMPA receptor number locally at synapses that undergo plastic changes and to adjust AMPA receptor-mediated glutamate signalling of acceptor nerve cells. The presence of glutamate receptors within neuron-secreted exosomes opens the possibility that other ion channels may also travel between neurons and influence their intrinsic properties (Fauré et al., 2006).

Concerning the communication between neurons and astrocytes, evidence was provided indicating that some EMV released by neurons are endocytosed preferentially by the astrocytes (Lizarraga-Valderrama and Sheridan, 2021). These neuronal exosomes cargoes may contain complementary combinations of proteins and miRNAs that help astrocytes to maintain homeostasis of neurotransmission in the CNS (Men et al., 2019). For instance, exosomes carrying miR-124a have been shown to be internalised by astrocytes. This causes an increase in astroglial glutamate transporter-1 protein expression levels in the target cells (Morel et al., 2013), a crucial protein for the homeostatic maintenance of glutamate levels in astrocytes and for preventing neuronal excitotoxicity (Yang et al., 2010).

This type of VT was found to be exploited by astrocytes as well (Venturini et al., 2019; Lizarraga-Valderrama and Sheridan, 2021), and astrocyte-released EMV contain a variety of cargos including proteins and RNAs that can modify the expression of proteins involved in signal decoding functions of target cells. It is noteworthy that exosomes released from the astrocyte processes proved the ability to selectively target neurons (Venturini et al., 2019). In this context, of particular interest are data indicating that the micro RNA miR-223 is particularly enriched in astrocytes and secreted by exosomes leading to a regulation of glutamate NMDA receptor in recipient neurons (Amoah et al., 2020). In ATP-stimulated primary astrocytes, EMV were identified containing SPARC, a protein able not only to promote axon outgrowth but also to regulate the level of postsynaptic AMPA receptors at maturing synapses of target neurons (Vincent et al., 2008). Of interest is also the possibility that astrocytic EMV could deliver cytokine receptors (such as $IL1R_2$, CCR_2 and $CXCR_4$) (Fuxe et al., 2013). Bioinformatics studies, indeed, suggested that these receptors may form heteroreceptor complexes with NMDA, GABA and D_2 receptors upon internalization, modulating their signaling properties (Borroto-Escuela et al., 2015, 2017b).

Evidence for intercellular GPCR transfer by exosomes at the protein and mRNA level has also been demonstrated in cell lines, as, for instance, U87MG cells from glioblastoma (Guescini et al., 2012; Woods et al., 2013). It involved adenosine A_{2A} and dopamine D_2 receptors. The EMV-mediated GPCR transfer resulted in the incorporation of functional receptors in acceptor cells which also may undergo A_{2A}-D_2 receptor heteromerization, as shown by photo-bleaching fluorescence resonance energy transfer.

Thus, EMV release in the roamer type of VT may represent a significant novel mechanism for the modulation of neuron-neuron and astrocyte-neuron intercellular signaling.

4. Concluding remarks and perspectives

Besides their well established support functions, increasing evidence indicates astrocytes as important elements for brain activity through signaling exchange with neurons, occurring in particular at synapses. Astrocytes, indeed, can sense synaptic activity thanks to a wide variety of neurotransmitter transporters and receptors they express. More importantly, their molecular machinery to respond to neurotransmitters (such as glutamate, GABA, acetylcholine, and norepinephrine) released by neurons involves Ca^{2+} elevations that, in turn, stimulate the release of neuroactive substances termed as gliotransmitters (e.g. glutamate, GABA, ATP/adenosine or D-serine) influencing nearby neurons (Araque et al., 2014; Durkee and Araque, 2019).

As briefly described in the previous sections, such a high diversity of signaling (Durkee and Araque, 2019), granting a variety of potential effects with significant functional consequences, is mediated by a quite large number of receptors expressed by both neurons and astrocytes. In this respect, however, a number of aspects still remain open to further investigation (Kofuji and Araque, 2021).

A first set of questions concerns the pattern of gliotransmitters released as a consequence of the action of a single neurotransmitter acting on a specific astrocytic receptor. Recently provided data (see (Covelo and Araque, 2018)) showed, for instance, that a single hippocampal astrocyte can release two gliotransmitters (ATP/adenosine and glutamate) in response to the stimulation of a single interneuron signaling to astrocytes through activation of $GABA_B$ receptors. Furthermore, the astrocytic glutamate- and ATP/adenosine-mediated effects on synaptic transmission depended on the duration and frequency of the interneuron stimulation, indicating that astrocytes are able to decode neuronal inputs and integrate this information into specific gliotransmitter release. The understanding of the relationship between neurotransmission and gliotransmission, however, is still limited and future research may certainly provide new and deeper findings on the topic.

In this context, a further aspect to consider are mechanisms modulating and integrating the different lines of bidirectional communication between astrocytes and neurons. Two specific mechanisms (RRI and EMV transfer of materials modulating the cell decoding apparatus) have been here briefly discussed.

The research effort to identify and characterize RRI has been mainly focused on neurons and clearly indicated this mechanism as a relevant factor contributing to set and tune the synaptic strength (Fuxe et al., 2010; Farran, 2017). This line of research allowed the identification of a high number of RRI, most presently stored in specific databases (see (Borroto-Escuela et al., 2014)). Available data on RRI and on receptor complexes in astrocytes are more limited. Furthermore, the analysis of such an issue could likely require a more detailed description of the cellular localization of the receptor complexes. This aspect, indeed, has been addressed only to a limited extent, with the majority of available studies being aimed only at demonstrating the presence of receptor complexes in the cells. This issue, however, could be of significant physiological importance, as indicated by the increasing number of studies revealing the existence of functional microdomains in astrocytes (see (Lia et al., 2021) for a recent review). The term “microdomains”

describes Ca^{2+} events that are restricted to small portions of individual astrocyte territories and can either remain restricted locally or eventually propagate to the main processes and to the soma of the cells. The characterization of the panel of receptors and receptor complexes associated with these sub-cellular functional domains could, therefore, represent a key step to increase our understanding of the astrocyte role in brain function. Thus, the study of the role played by RRI in neuron-astrocyte signaling is probably still at the beginning. Although further studies are needed to expand this knowledge, the available data seem to indicate that the bidirectional astrocyte-neuron signaling at synapses may find in RRI a significant regulatory mechanism giving high flexibility to intercellular communication at this level (Guidolin et al., 2021). In this respect, a further line of future investigation, potentially providing significant additional information, could be focused on possible structural differences between the conformation assumed by receptors and receptor complexes in neurons and astrocytes as a consequence of the differences of membrane potential between the two cell types (McNeill et al., 2021). Differences in the energy landscape, indeed, modulate the pattern of allosteric interaction between monomers and may lead to changes in the signaling features of the complex they can form (Guidolin et al., 2011).

The possible relevance for the intercellular communication of the EMV-mediated exchange of materials modulating the cell recognition apparatus can be appreciated in the light of several experimental findings obtained in a variety of cell types. Of particular interest is evidence demonstrating that receptors can be transferred from one cell to another via the exosome pathway. Examples include bystander B cells acquiring antigen receptors from activated B cells (Quah et al., 2008) and TfR2 found to be part of the exosomal budding vesicles in erythroleukemic and hepatoblastoma cells (Calzolari et al., 2006). In particular, these authors demonstrated that TfR2 localizes in low-density plasma membrane microdomains, where it promotes cell signaling, and is exported out of the cells through exosomes, where it acts as an intercellular messenger. For what it concerns receptors involved in the recognition/decoding of signals by neurons and astrocytes available data have been here summarized. Although still quite limited, the available experimental evidence suggests that this mode of intercellular communication can lead to a modulation of the target cell decoding apparatus or to the transient acquisition by the target cell of the capability to recognize/decode transmitters for which the cell does not express the pertinent cognate receptors. Future research on EMV release in the roamer type of VT may therefore provide a deeper understanding of this novel, potentially important, mechanism modulating neuron-astrocyte cross-talk.

The views emerging from the study of intercellular signaling in the CNS may also significantly impact on neuropharmacology. The classical view of synapse, indeed, is still the most followed reference framework on which pharmacological approaches to neurological disorders are based (Webster, 2001). However, current strategies often have limited efficacy at best and the most obvious reason is that the classical view does not consider the full network of interactions and signals to select the best target to address symptoms and/or disease progression without triggering deleterious side effects. In this respect, the new views on the intercellular communication in the CNS emerged in the last decades and leading to a broader view of the synaptic dynamics (tripartite/pentapartite synapse) may offer new perspectives to target the problem (Guidolin et al., 2022), as, for instance, exploring novel, glia-mediated strategies to address neurodegenerative and functional (Franco et al., 2019; Hernandez-Sosa et al., 2020) CNS disorders.

In particular, the here discussed mechanisms modulating intercellular signaling may represent targets of particular interest. In this respect, receptor complexes emerging from allosteric RRI are the most explored field to design new pharmacological strategies for the treatment of CNS pathologies (Guidolin et al., 2022). The most followed approach to target receptor complexes has been the well designed use of agonists/antagonists of a given protomer, based on the fact that the pharmacology of some agonists/antagonists of a given protomer may show substantial differences among different receptor complexes in terms of affinity and efficacy. In this context, it is of interest to mention the recent approval in the United States of an A_{2A} antagonist (istradefylline), targeting the A_{2A}-D_2 heterodimer, as an adjunctive treatment to L-DOPA in patients with PD experiencing "off" episodes (see Guidolin et al., 2020 for references). The development, however, of bivalent ligands (Daniels et al., 2005) or of allosteric modulators selective for structural domains in the heteroreceptor complexes (Agnati et al., 2006; Cervetto et al., 2018) appears as a further very promising strategy. In view of their natural ability of transferring biological information, EMV also became a highly attractive candidate for therapeutic delivery vehicle in neurodegenerative diseases. A number of studies in experimental models of brain injury, indeed, provided significant preliminary evidence that EMV could be used to help neuroprotection (see Lee and Kim, 2017). Examples include the reduced deposition of amyloid-β induced by intracerebrally administered neuroblastoma-derived exosomes and its clearance by microglial cells (Yuyama et al., 2015), and the reduced inflammation of ischemic areas in a rat model of stroke following systemic administration of mesenchymal cell-derived EMV (Xin et al., 2013).

Data availability

No data was used for the research described in the article.

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