

Review

Regulation of cell-to-cell communication mediated by astrocytic ATP in the CNS

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Abstract

It has become apparent that glial cells, especially astrocytes, not merely supportive but are integrative, being able to receive inputs, assimilate information and send instructive chemical signals to other neighboring cells including neurons. At first, the excitatory neurotransmitter glutamate was found to be a major extracellular messenger that mediates these communications because it can be released from astrocytes in a Ca^{2+} -dependent manner, diffused, and can stimulate extra-synaptic glutamate receptors in adjacent neurons, leading to a dynamic modification of synaptic transmission. However, recently extracellular ATP has come into the limelight as an important extracellular messenger for these communications. Astrocytes express various neurotransmitter receptors including P2 receptors, release ATP in response to various stimuli and respond to extracellular ATP to cause various physiological responses. The intercellular communication “ Ca^{2+} wave” in astrocytes was found to be mainly mediated by the release of ATP and the activation of P2 receptors, suggesting that ATP is a dominant “gliotransmitter” between astrocytes. Because neurons also express various P2 receptors and synapses are surrounded by astrocytes, astrocytic ATP could affect neuronal activities and even dynamically regulate synaptic transmission in adjacent neurons as if forming a “tripartite synapse”. In this review, we summarize the role of astrocytic ATP, as compared with glutamate, in gliotransmission and synaptic transmission in neighboring cells, mainly focusing on the hippocampus. Dynamic communication between astrocytes and neurons mediated by ATP would be a key event in the processing or integration of information in the CNS.

Abbreviations: $[\text{Ca}^{2+}]_i$ – intracellular Ca^{2+} concentration; CFTR – cystic fibrosis transmembrane conductance regulator; CNS – central nervous system; InsP_3 – inositol 1,4,5-trisphosphate; SNARE – soluble *N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptor

Introduction

In the mid-19th century, Rudolph Virchow, a German anatomist, first found non-neuronal cells in the central nervous system (CNS) and called them “glia”, a *Greek* for “glue”. The name reflects the original view that glia played merely a structural or supportive role for neurons. They occupy over 70% of the total cell population in the CNS and are classified into microglia, oligodendrocytes and astrocytes. Now it has become apparent that glia, especially astrocytes, are much more than “glue” but rather are integrative, being able to receive inputs, to assimilate

information and to send instructive chemical signals both to neurons and to other neighboring cells. Although rapid neurotransmission was believed to be restricted solely to neuron-to-neuron communication, it has been found to include glial cells [1, 2]. The first evidence for dynamic communication from astrocytes to neurons came from the discovery of temporally related changes in the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in glial and neuronal cells. Various stimuli which selectively elevate $[\text{Ca}^{2+}]_i$ in astrocytes lead to delayed elevations in $[\text{Ca}^{2+}]_i$ in neurons in culture [3]. In hippocampal slice preparations, activation of metabotropic glutamate receptors in astrocytes evokes Ca^{2+} signals in astrocytes, which are followed by a delayed elevation of neuronal Ca^{2+} levels [4, 5]. Evidence suggests that such Ca^{2+} -mediated extracellular signaling between astrocytes and neurons may be involved in the regulation of synaptic transmission. Stimulation of Ca^{2+} waves in

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astrocytes can increase both excitatory and inhibitory postsynaptic currents in hippocampal cultures [6]. In the retina, astrocytic Ca^{2+} waves can modulate the light-induced excitation of ganglion cells [7]. Glutamate appears to be an important mediator for these astrocyte-to-neuron signals.

There is an increasing body of evidence however, that ATP, the predominant extracellular signaling molecule among astrocytes [8–12], may also mediate signaling between neurons and glial cells [13]. Neurons are known to express a wide variety of ionotropic (P2X) and metabotropic (P2Y) receptor subtypes in the pre- and postsynaptic regions, and ATP could directly mediate synaptic transmission as a fast neurotransmitter in the rat medial habenula [14] and in the spinal cord dorsal horn [15]. In addition, exogenously applied ATP potentiates [16–19] or inhibits [20, 21] synaptic transmission in the CNS. Given that astrocytic Ca^{2+} waves can evoke changes in neuronal synaptic activity and that Ca^{2+} waves are mediated by the release of ATP, ATP released from astrocytes may be involved in astrocyte-to-neuron signaling in synaptic regions of the CNS.

In this review, we summarize the role of astrocytic ATP, as compared with glutamate, in gliotransmission and synaptic transmission in neighboring cells, mainly focusing on the hippocampus. This finding of a novel ATP-mediated signaling system between astrocytes and neurons complements a growing body of evidence, suggesting that, in addition to their various supportive roles for neurons, astrocytes are actively involved in the control of synaptic transmission.

Astrocyte-to-astrocyte communication “ Ca^{2+} wave”

The development of video imaging techniques allowed the observation that neurotransmitters elicit increases in $[\text{Ca}^{2+}]_i$ even in glial cells. Since unlike neurons, astrocytes do not produce action potentials, they were thought to be quiet. However, they have rather found to be busy or noisy in terms of “ Ca^{2+} excitability”. About 15 years ago, elevations in $[\text{Ca}^{2+}]_i$ in individual cultured astrocytes in response to neurotransmitters were first reported [22–24]. After initial observations demonstrated the presence of Ca^{2+} excitability within astrocytes, it became apparent that many neurotransmitters stimulate Ca^{2+} elevations in glial cells by activating specific receptors expressed on these cells. Astrocytes express metabotropic glutamate receptors [3], dopamine receptors [25, 26], noradrenaline receptors [27], serotonin receptors [28–31] and P2 receptors [8, 9, 32–36], whose activation results in elevations in $[\text{Ca}^{2+}]_i$ astrocytes. Subsequently, it was demonstrated that these Ca^{2+} elevations could in turn stimulate the release of chemical transmitters from glial cells, which mediates a communication between astrocytes and even neurons (see other section). Cornell-Bell (1990) showed that glutamate can elicit $[\text{Ca}^{2+}]_i$ not only in individual cells, but also intercellular waves of increased $[\text{Ca}^{2+}]_i$ that are propagated from single cells to multiple neighboring cells [24]. Dani et al. (1992) showed that neuronal activity can directly initiate such a Ca^{2+} wave in astrocytes [37]. Other stimuli

such as local mechanical or electrical stimulation were subsequently observed to initiate similar intercellular Ca^{2+} signaling in astrocytes. Mechanical stimulation with a micropipette reliably evokes spreading Ca^{2+} waves in astrocytes [38], and has been used extensively as a stimulus for the focal initiation of Ca^{2+} waves, allowing us to analyze their characteristic spatiotemporal features. To evoke Ca^{2+} wave, the mechanical stimulus need not be associated with cell damage, since repetitive stimulation of the same cell can evoke repetitive Ca^{2+} waves with recovery of the $[\text{Ca}^{2+}]_i$ to baseline levels between stimuli and with no leakage of intracellular Ca^{2+} indicator dyes. For some years, such Ca^{2+} waves have been thought to propagate via gap junctions [39–42], through which the internal messenger inositol 1,4,5-trisphosphate (InsP_3) can be diffused to mobilize Ca^{2+} release [42, 43]. Stimulating a single glial cell leads to the production of InsP_3 , triggering the release of Ca^{2+} from internal stores in the stimulated cell as well as in adjacent cells. More recently, experiments in culture have shown that Ca^{2+} waves can be propagated between astrocytes, even when the cells do not contact each other directly, and the extent and direction of the Ca^{2+} wave propagation are significantly influenced by movement of the extracellular medium [8, 44]. Subsequent publications have confirmed that astrocytes do not absolutely require functional gap junction coupling for the spreading of Ca^{2+} waves in astrocytes [45, 46]. These more recent reports suggest that substances released from astrocytes can activate receptor systems on astrocytes, evoking the release of additional substances (either the same or different compounds), and thus producing a propagating Ca^{2+} wave of activity. Recently, it has been found that extracellular ATP is the major messenger for this event. First, ATP is released from astrocytes during Ca^{2+} wave propagation [8, 11]. Second, the propagation can be reduced or abolished by a purinergic antagonist [8, 10–12, 35, 47] or the ATP degrading enzyme apyrase [8, 47, 48]. In addition, visualization of the release of ATP demonstrated that the velocity of ATP release well correlates with that of the Ca^{2+} wave in astrocytes [47]. All these findings suggest that the extracellular molecule ATP could be a primary signal for the Ca^{2+} wave propagation, and highlight the importance of ATP in cross-talk among astrocytes and even other cell types in the CNS.

So far, the physiological consequences of the ATP-evoked increase in $[\text{Ca}^{2+}]_i$ in astrocytes themselves have received only limited attention. Stimulation of astrocytes with ATP enhances mitogenic signaling via the ERK-mediated pathway, increases proliferation [49, 50], and protects astrocytes against oxidative stress [51]. Further comprehensive studies will reveal the importance of ATP-mediated Ca^{2+} responses in astrocytes.

Astrocyte-to-microglia communication

Intercellular Ca^{2+} waves in astrocytes also trigger microglial Ca^{2+} responses in a manner dependent on extracellu-

lar ATP. As described above, astrocytes release ATP upon mechanical stimulation [8], electrical stimulation [8] or glutamatergic receptor activation [52], and respond to locally applied ATP with a propagating Ca^{2+} wave. Microglia have been shown to express functionally active P2 receptors in culture and *in situ* [53–60], suggesting the possibility that ATP could also mediate astrocyte-to-microglia communication. In fact, Verderio and Matteoli demonstrated that mechanical and bradykinin stimulation resulted in the release of ATP from astrocytes, which, in turn triggered delayed Ca^{2+} responses in adjacent microglia via P2X7 receptors in a mixed culture of astrocytes and microglia [61]. Schipke et al. showed that electrical stimulation of astrocytes produced Ca^{2+} waves that triggered Ca^{2+} responses in microglia in an extracellular ATP-dependent manner in a slice preparation of mouse. Thus, it appears that extracellular ATP-dependent Ca^{2+} waves could occur *in situ* and are not restricted to astrocytes but broadly activate different glial cell types. The astrocytic ATP-mediated intercellular communication was also observed in meningeal cells [62] and Muller cells [35]. Recently, Tsuda et al. have reported that the expression of P2X₄ receptors in the spinal cord is enhanced in spinal microglia after peripheral nerve injury, and that blocking pharmacologically and suppressing molecularly P2X₄ receptors cause a reduction of the neuropathic pain behavior [55, 63]. Thus, P2-receptor mediated microglial Ca^{2+} excitability may be of great consequence for pathological events such as chronic pain. In addition, peripheral sensory axons release ATP that activates P2 receptors in neighboring Schwann cells, leading to the spreading of Ca^{2+} waves in the cells and regulation of their proliferation/differentiation [13]. It seems that extracellular ATP may function as a ubiquitous autocrine/paracrine in central and peripheral tissues.

Mechanisms of ATP release from astrocytes

Although several excitable and non-excitable cells release ATP, the mechanism underlying the release of ATP is controversial, especially in non-excitable cells such as astrocytes. With regard to glutamate release from astrocytes, recently some important findings have been reported. Astrocytes express SNARE proteins [64–67], have a vesicular structure expressing the vesicular glutamate transporters [65, 66], and the release of glutamate is dependent on Ca^{2+} [68, 69], sensitive to SNAREs [65, 67, 70]. These findings strongly suggest that exocytotic machinery is involved in glutamate release in astrocytes although non-vesicular mechanisms for glutamate release are also proposed [71–74]. In contrast, mechanisms underlying the release of ATP from astrocytes are still a matter of debate. The release of ATP is reduced by inhibitors for several anion channels [75, 76], ATP binding cassette proteins or CFTR [77–81], gap junction [10, 82], suggesting the involvement of multiple pathways for the release. In addition, the release of ATP is partly dependent

on Ca^{2+} [10, 79, 83], and SNARE proteins [84, 85], and astrocytes seem to possess vesicles that contain ATP inside [84, 86]. Inhibition of ATP release by vesicular ATPase inhibitors was also reported [86]. These findings suggest that the mechanisms of ATP release could include exocytosis. Furthermore, the nature of the signals released from astrocytes may differ under varying physiological and pathological conditions [86]. Exocytotic mechanisms for ATP have been reported in other non-excitable cells. For example, in vascular endothelial cells, the shear stress-evoked ATP release is vesicular and dependent on Ca^{2+} [87]. It would be very important to elucidate the mechanisms by which astrocytes release ATP in response to distinct stimuli, which would further establish the position of astrocytes as an important partner of neurons in forming the “tripartite synapse” [88].

Astrocyte-to-neuron communication

As described above, astrocytes lack the ability to propagate regenerative electrical signals but are nonetheless responsive to a variety of extracellular stimuli and produce regenerative Ca^{2+} waves that spread within astrocyte networks [8, 12, 35, 47]. Ca^{2+} excitability in astrocytes can evoke the release of neuroactive substances such as glutamate and ATP.

Glutamate is the predominant signaling molecule among the previously reported mechanisms through which astrocytes can actively regulate synaptic transmission. In cultured hippocampal neurons, the stimulation of astrocytes evokes a regenerative, Ca^{2+} -dependent release of glutamate from astrocytes which, in turn, can enhance excitatory synaptic transmission via *N*-methyl-D-aspartate receptor-mediated mechanisms [6]. Glutamate-mediated astrocyte-to-neuron signaling has also been observed in hippocampal slices [89–91], visual cortical slices [3] and in the retina [7], although the subclass of responsible glutamate receptors varied among the different preparations. However, astrocytic ATP has recently been shown to decrease the excitability of neurons in the retina [92], and mediate presynaptic inhibition in cultured hippocampal neurons [47, 93]. Cultured hippocampal neurons reveal synchronous spontaneous Ca^{2+} oscillation, which is extracellular Ca^{2+} -dependent, tetrodotoxin-sensitive and inhibited by inhibitors of ionotropic glutamate receptors, suggesting that the neuronal Ca^{2+} oscillation is mediated by glutamatergic synaptic transmission [20, 47, 94, 95]. Endogenous ATP released from astrocytes dynamically downregulates the spontaneous neuronal Ca^{2+} oscillation [47] and EPSCs in the hippocampal culture [93] by inhibiting presynaptic functions of glutamatergic neurons. Similar astrocytic ATP-mediated presynaptic inhibition was observed in the hippocampus *in situ* [93] although adenosine, a metabolite of ATP degraded by ecto-nucleotidases, also functioned as an inhibitory molecule in the slices. ATP would differ from glutamate as a signaling molecule between astrocytes and neurons in that it inhibits rather than potentiates synaptic

transmission. We hypothesize that the opposing actions of glutamate and ATP released from astrocytes represent a means by which astrocytes can dynamically modulate neuronal activity by releasing distinct transmitters which can either excite or inhibit synaptic transmission. Very recently, Bowser and Khakh (2004) have demonstrated that electrical stimulation of Schaffer collaterals and perforant path in hippocampal slices evoked both an increase in $[Ca^{2+}]_i$ in astrocytes and facilitation of GABAergic IPSCs onto interneurons in the CA1 stratum radiatum [96], both of which were mediated by P2Y1 receptors. Similar responses were observed in the CA3 region of the hippocampus [97]. Endogenously released ATP from neurons and astrocytes can activate P2Y1 receptors on astrocytes and postsynaptic interneurons to activate $[Ca^{2+}]_i$ elevations and facilitation of synaptic inhibition, respectively. This might be another form of astrocyte-to-neuron communication in the hippocampus because astrocytes may release and propagate a wave of ATP, which activates P2Y1 receptors on postsynaptic GABAergic interneurons, leading to increased synaptic inhibition in interneuron networks *in situ* [96, 97].

In addition to mediating inhibitory rather than excitatory effects on synaptic transmission, ATP-mediated astrocyte-to-neuron signaling further differs from glutamate-dependent signaling mechanisms by the fact that it occurs in a tonic fashion [47, 93]. Application of the ATP-degrading enzyme apyrase induces a potentiation of spontaneous neuronal Ca^{2+} oscillations or EPSCs in the absence of any astrocytic stimulation, suggesting the presence of a constitutive ATP-dependent inhibition of synaptic transmission. Furthermore, spontaneous astrocytic Ca^{2+} responses occur in both purified astrocyte cultures and mixed cultures of astrocytes and neurons. The spontaneous Ca^{2+} signals in astrocytes were inhibited by apyrase but persisted in the presence of TTX. Therefore, astrocytes constitutively release ATP in the absence of neuronal activity, which exerts tonic down-regulation of excitatory synaptic transmission [47, 93]. ATP mediates astrocytic Ca^{2+} waves and can evoke neuronal Ca^{2+} responses in various parts of the CNS such as the habenula [14], suggesting that ATP may be an ubiquitous mediator of astrocyte-to-neuron signaling in the modulation of synaptic activity. Such a tonic modulation by astrocytic ATP might be a mechanism by which neurons tune their communications in the CNS.

The ATP receptor subtype(s) implicated in the ATP-mediated inhibition of presynaptic transmission in hippocampal neurons remains unknown [47, 93]. Although apyrase abolished the ATP-mediated inhibition, the non-selective P2 receptor antagonists suramin and PPADS were only able to partially attenuate the effects of exogenously applied ATP [20]. Similarly, these antagonists only slightly affected the decrease in neuronal Ca^{2+} oscillations evoked by mechanical stimulation of an astrocyte [47] but reactive blue 2 reduced the effect of ATP [93]. Thus, involvement of P2Y receptors in the inhibitory action was suggested [93] although reactive blue 2 could also affect P2X receptors. Adenosine, a metabolite of ATP, is also involved in the inhibitory action via adenosine A1 receptors [20, 21,

98]. Released ATP might exhibit its inhibitory action by being metabolized into adenosine. However, the ATP-evoked inhibition did not disappear even in the presence of several antagonists to adenosine receptors, A1 receptors or adenosine deaminase in hippocampal neurons [20, 99, 100]. In addition, the effect of astrocytic ATP on the synaptic transmission almost disappeared in the presence of apyrase (grade III), which degrades ATP and ADP into ADP and AMP, respectively but does not affect the metabolism of adenosine [20, 47]. All these findings suggest that ATP itself is involved in the inhibition of synaptic transmission, but we cannot identify the responsible receptor subclasses so far. Recently, the oligomeric association of A1 receptors with P2Y1 receptors (A1/P2Y1 receptors) generating A1 with P2Y1 receptor-like agonistic pharmacology has been reported [101, 102]. Such an oligomeric association occurs in hippocampal neurons [103] and the pharmacological characteristics of A1/P2Y1 receptors are similar to those involved in the inhibition of neuronal Ca^{2+} oscillations [20]. However, the discovery of specific antagonists for such oligomeric A1/P2Y1 receptors is required to determine if they are involved in ATP-mediated inhibition of neuronal activity.

Conclusion

Astrocytes release ATP, glutamate or other active substances in response to various stimuli or even spontaneously, by which the activities of adjacent astrocytes or even neurons are positively and dynamically controlled (Figure 1). Now we know that rapid neurotransmission is not restricted solely to neuron-to-neuron communication but also includes glial cells. Especially, astrocytes can receive neurotransmitters, respond to them, and send output signals to neighboring neurons, forming a so-called "tripartite synapse". For this, extracellular ATP and P2 receptors appear to have a central role. Further extensive

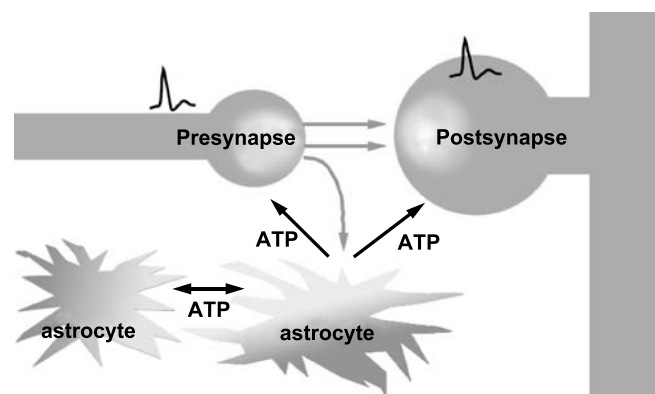


Figure 1. A schematic diagram of "tripartite synapse" mediated by extracellular ATP neurotransmitters, spilling over from the synapse, stimulate surrounding astrocytes (gray arrows). Astrocytes have a big variety of neurotransmitter receptors, and can release diffusible molecules such as ATP in response to the neurotransmitters, and form propagating Ca^{2+} waves in astrocytes. Astrocytic ATP has an essential role for the formation of the intercellular Ca^{2+} wave, and in turn functions as a feedback signal to modulate synaptic transmission in adjacent neurons.

studies will be required to clarify the physiological or pathological significance of astrocytes in synaptic transmission, the mechanisms underlying the release of ATP from astrocytes and the distinct functions of glutamate and ATP as gliotransmitters. At present, it may be thought that glial cells play a subordinate role in the brain function, especially in the processing and integration of information. However, the glial era has just started, and exciting discoveries can be expected. Being partner with ATP, astrocyte can be a star.

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