Review

Author for correspondence:
Dustin J. Hines
e-mail: dustin.hines@tufts.edu

Astrocytic adenosine: from synapses to psychiatric disorders

Dustin J. Hines and Philip G. Haydon

Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA

Although it is considered to be the most complex organ in the body, the brain can be broadly classified into two major types of cells, neuronal cells and glial cells. Glia is a general term that encompasses multiple types of non-neuronal cells that function to maintain homeostasis, form myelin, and provide support and protection for neurons. Astrocytes, a major class of glial cell, have historically been viewed as passive support cells, but recently it has been discovered that astrocytes participate in signalling activities both with the vasculature and with neurons at the synapse. These cells have been shown to release D-serine, TNF-α, glutamate, atrial natriuretic peptide (ANP) and ATP among other signalling molecules. ATP and its metabolites are well established as important signalling molecules, and astrocytes represent a major source of ATP release in the nervous system. Novel molecular and genetic tools have recently shown that astrocytic release of ATP and other signalling molecules has a major impact on synaptic transmission. Via actions at the synapse, astrocytes have now been shown to regulate complex network signalling in the whole organism with impacts on respiration and the sleep–wake cycle. In addition, new roles for astrocytes are being uncovered in psychiatric disorders, and astrocyte signalling mechanisms represents an attractive target for novel therapeutic agents.

1. Introduction

Astrocytes have historically been viewed as passive support cells, but recently it has been discovered that astrocytes participate in signalling activities both with the vasculature and with neurons at the synapse. However, the concept of astrocytes as active participants is not entirely new: in 1895 Santiago Ram'on y Cajal proposed that astrocytes control sleep and waking states [1]. Cajal hypothesized that astrocytic processes act as insulators surrounding neurons to facilitate sleep, and then retracting to allow neuronal communication facilitating wakefulness [2]. A century of research since the time of Cajal has provided support for parts of his original suggestion. We now know that astrocytes have both structural and functional links with neurons and via these links are able to modulate complex behaviours including sleep [3] and contribute to disorders of the brain including depression [4].

2. Astrocyte signalling

As mentioned above, Cajal systematically studied astrocytes from a structural standpoint [2], and until very recently, our understanding of astrocytic morphology has been largely based on Cajal's metal impregnation methods, or on glial fibrillary acidic protein (GFAP) staining. These methods both permit static endpoint assessment of cells, providing only a snapshot of their structure and function. Novel labelling and imaging technologies have revealed that the structure of astrocytes is far more complicated than previously appreciated. In conjunction with GFAP staining, researchers filled astrocytes with fluorescent dyes, revealing that GFAP staining only represented 15% of the true astrocytic volume and that astrocytes extend numerous fine processes virtually filling the surrounding neuropil [5]. These studies also revealed that astrocytes occupy non-overlapping spatial territories, and that a single astrocyte contacts

hundreds of neuronal processes and multiple neuronal cell bodies within these territories [5].

In addition to their contact with neurons, astrocytes are known to line the vasculature with their endfeet [6]. This position between neurons and blood vessels allows astrocytes to mediate neurovascular coupling, the process by which neuronal activity and metabolic demands are coupled to blood flow. Astrocytes play essential roles in brain energy homeostasis and metabolism which is again mediated by their close link with the vasculature, and astrocytes are known to express the machinery required for the uptake of glucose from blood vessels.

By controlling the metabolic and ionic milieu surrounding neurons, astrocytes can dramatically impact neuronal activity. The numerous processes of a single astrocyte contact tens of thousands of synapses, and examinations of diverse brain regions have shown that up to 50% of excitatory synapses are closely coupled to an astrocytic process [7]. This close connection between synapses and astrocytic processes is both structural and functional, and has been termed the tripartite synapse (figure 1) [8]. Evidence from many groups of researchers now supports the concept that astrocytes act as both 'listening' and 'talking' participants in the tripartite synapse via multiple regulated signalling pathways [9].

Recent attention has turned to how astrocytes may actively be sending signals to the neurons to which they are coupled, as opposed to passively monitoring the signalling activities of neurons. In addition to the established roles of astrocytes in controlling the ionic environment of the neuropil and controlling the supply of neurotransmitters to synapses, astrocytes are now known to regulate neuronal signalling by direct communication via regulated release of signalling molecules. Astrocytes have now been shown to signal via many chemical transmitters, including classical transmitters, peptides, chemokines and cytokines, and they achieve this diverse communication through a number of different release mechanisms. The varied signalling systems of astrocytes can have complex downstream effects on neurons. For example, glutamate and D-serine released from astrocytes enhances N-methyl-D-aspartate (NMDA) receptor-mediated current and results in excitatory feedback to neurons [10], whereas the release of ATP from astrocytes (converted to extracellular adenosine and acting on presynaptic adenosine A_1 receptors) ultimately results in inhibition of synaptic transmission [11].

Multiple mechanisms and modes of release have been proposed for astrocytes signals, and it is thought that these may operate under different physiological or pathological contexts. Studies have described connexin/gap junction [12–14], volume-regulated anion channel [15,16] and exocytotic release [17–19] mechanisms operating in astrocytes.

Figure 1. The tripartite synapse composed of physically and functionally interacting synaptic terminals and astrocyte processes. The electron micrograph on the left shows a hippocampal synapse surrounded by astrocyte process end feet. On the right, outlines of the components of the tripartite synapse clearly show the astrocytes end feet (dark grey) tightly surrounding the presynaptic and postsynaptic compartments (light grey), providing a tight seal around the sight of neurotransmission.

assemble into tight, four-helix bundles called 'trans'-SNARE complexes. Core SNARE complex machinery proteins are expressed in astrocytes, including synaptobrevin II [20,21] and its homologue cellubrevin [22] and SNAP-23 [20,21]. Ancillary proteins for exocytosis are also expressed in astrocytes, such as Munc 18 [20,23], complexin 2 [20,21] and synaptotagmin IV [23].

Beyond molecular identification of SNARE protein expression, astrocytes have been shown to possess vesicular structures. Electron microscopic studies from tissue have revealed the existence of clear astrocytic vesicles of appropriate diameter in the vicinity of presynaptic terminals [24], which strongly supports the existence of a vesicular pathway of gliotransmission in the intact brain. SNARE proteins have been shown to colocalize with a number of vesicular organelles in cultured astrocytes, including small vesicles positive for vesicular glutamate transporters (VGlut 1–3) [20–23], ATP-storing vesicles [18,25], and D-serine-containing vesicles [26], suggesting the involvement of vesicular mechanisms in the release of these signalling molecules from astrocytes.

A turning point in our understanding of astrocyte signalling resulted from calcium imaging studies showing that cultured astrocytes release glutamate and lead to elevation of calcium in nearby neurons [27]. Several subsequent studies demonstrated the occurrence of this process in acute brain slices [28–33] and in vivo [34]. Since these pioneering studies, astrocytes have been shown to release a number of chemical transmitters, including ATP [35–37], D-serine [10,38], TNF-α [39] and atrial natriuretic peptide (ANP) [40], in a process that is now collectively termed gliotransmission.

3. Astrocytes signal via exocytosis

Exocytosis is a well-characterized process of release that is known to occur in multiple cell types. Exocytosis is a regulated process that depends upon docking and fusion of vesicles to the plasma membrane, achieved by the formation of the soluble NSF (N-ethylmaleimide-sensitive fusion) protein **a**ttachment protein **re**ceptor (SNARE) complex. Proteins that make up the SNARE complex are small, abundant and are primarily membrane bound. Despite diverse structures, all SNARE proteins share a segment in their cytosolic domain called a *SNARE motif* consisting of 60–70 amino acids that
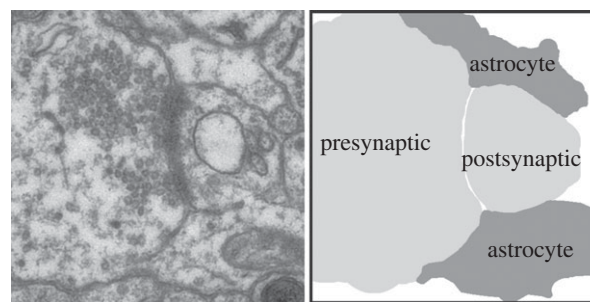
4. ATP gliotransmission

ATP is a major extracellular messenger that coordinates the function of astrocytes and communication between them and other cell types [14]. The mechanism by which astrocytes release ATP is not completely understood, but support for an exocytotic mechanism has emerged. In particular, electrophysiological studies have shown calcium-dependent changes in the area of the plasma membrane in single astrocytes, reflecting calcium-regulated vesicle fusion. Quinacrine binding of ATP in peptidergic vesicles has
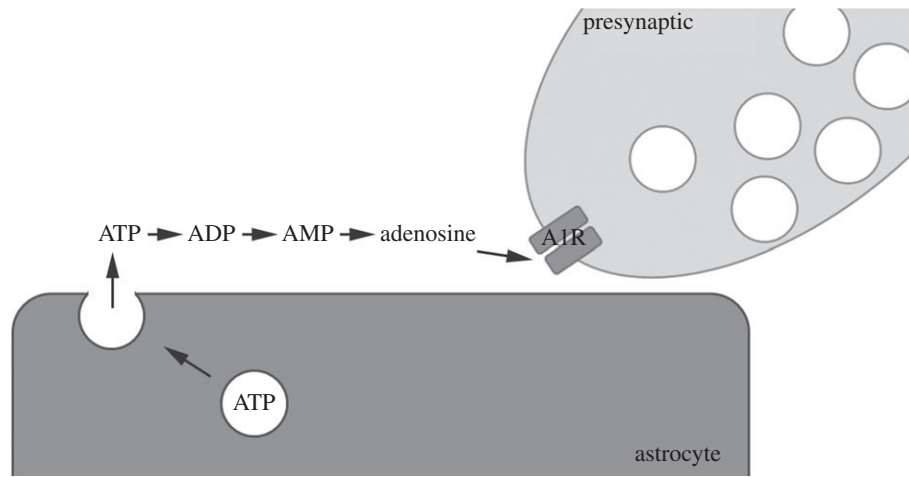
Figure 2. ATP signalling via exocytic release from astrocytes. Upon activation/stimulation astrocytes release ATP via exocytosis. In the extracellular space, ATP is hydrolysed to adenosine, which is then free to act on adenosine receptors. One major site of action is on A1 receptors in the presynaptic terminal.

shown that ATP is stored in secretory vesicles with peptides such as ANP within astrocytes [19]. In these studies, ionomycin treatment decreased the total image fluorescence and the number of quinacrine-stained vesicles, suggesting exocytosis of these vesicles following treatment [19]. Other studies have recorded from HEK-293 T cells transfected with a mutated purinergic receptor $P2X_3$ (D266A) that has reduced desensitization while retaining receptor affinity [41]. In D266A expressing HEK-293 T cells neighboured by astrocytes, small, transient, inward currents (STICs) with kinetic properties suggestive of quantal release could be detected [19]. Addition of glutamate to stimulate astrocytes increased the average frequency of STICs in expressing HEK-293 T cells. Because HEK-293 T cells do not respond to glutamate directly, it can be assumed that the increased frequency of recorded STICs resulted from astrocyte release. Calcium free solution reduced STICs in both resting and glutamate-stimulated conditions consistent with the requirement of calcium for SNARE mediated exocytosis. Recent research by Lalo et al. [42] has shown a significant difference in the baseline amplitude of miniature inhibitory currents in wild-type and dn-SNARE mice. This shows conclusive evidence that vesicular release of gliotransmitters may be involved in the long-term homeostatic regulation of inhibitory neurotransmission [42].

Other studies have shown reduction in the release of ATP by inhibitors of anion channels [16], ATP-binding cassette proteins or cystic fibrosis transmembrane conductance regulator [43], gap junctions [13] and $P2X_7$ receptors, which suggests the involvement of multiple pathways in ATP release from astrocytes.

ATP hydrolysis is well understood to lead to the accumulation of adenosine; however, the origin and mechanism of adenosine accumulation in the brain was not clearly revealed until the seminal study of Pascual et al. [44]. These studies used a molecular genetic strategy to perturb gliotransmission via conditional, astrocyte-specific expression of a dominant negative inhibitor of SNARE-dependent membrane fusion (the cytoplasmic tail of synaptobrevin 2; dnSNARE). Recordings from the hippocampal Schaffer collateral-CA1 synapses in mice expressing dnSNARE revealed enhanced synaptic transmission compared to wild-type littermates, or transgenic mice in which transgene expression was prevented by doxycycline in the rodent chow [44]. Also, it has been noted [45] that dnSNARE mice, and mice injected with dnSNARE

virus do not show alterations in astrocyte morphology from wild-type controls. These studies went on to show that blocking exocytosis from astrocytes using dnSNARE reduced ATP and its metabolite adenosine, which would normally exert tonic suppression of synaptic transmission.

5. Astrocytic regulation of ATP at synapses

Release of ATP from astrocytes has now been shown to be important for modulation of multiple neuronal signalling pathways with implications for behavioural output. For example, in hypothalamic slices, release of ATP from astrocytes is both necessary and sufficient for noradrenaline-dependent synaptic potentiation [46]. Following adrenergic stimuli, signalling via α1-adrenergic receptors expressed on astrocytes initiates release of ATP onto nearby magnocellular neurosecretory neurons. In turn, ATP activates $P2X_7$ receptors on these neurons, enhancing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor surface expression and the amplitude of miniature excitatory postsynaptic currents [46]. Experiments conducted in the retina have shown a suppression of neuronal activity resulting from astrocytic purinergic signalling [47–49]. Stimulation of photoreceptors in retinal wholemounts leads to glial calcium signalling [48,50] and subsequent ATP release from Müller cells [37,51]. Released ATP is degraded to adenosine, which then acts on A_1 receptors to suppress neuronal activity (figure 2). Similarly in the hippocampus, suppressive actions of astrocyte-derived adenosine have been observed via A_1-dependent presynaptic inhibition of synaptic transmission [44,52]. Astrocytes and neurons share many similar receptors. Because of this, they use similar signalling pathways, making it difficult to use pharmacological manipulations to distinguish the specific role of astrocytes in the modulation of neurons. Astrocyte-derived ATP-adenosine signalling has been confirmed using glia-specific toxins [52] and astrocyte-selective loading of the calcium chelator BAPTA as well as astrocyte-specific molecular genetics [53].

6. Astrocytic regulation of adenosine in sleep

Activation of the A1 receptor has previously been shown to regulate homeostatic functions of sleep [54]. Adenosine accumulates in the brain with prolonged periods of

wakefulness [55]. The selective addition of adenosine directly into the brain induces sleep [56,57] and electrophysiological markers of homeostatic sleep pressure [58], whereas antagonizing adenosine by pharmacological agents promotes wakefulness [59] and attenuates the accumulation of homeostatic sleep pressure [60]. One of the most common indications that adenosine signalling is implicated in the control of human sleep comes from the powerful wake promoting effects of adenosine receptor antagonists including caffeine [61]. In addition, humans with polymorphisms in the adenosine metabolizing enzyme, adenosine deaminase, show reduced adenosine metabolism and exhibit more consolidated sleep [62,63].

Sleep and sleep disorders are highly correlated with many psychiatric disorders, and more than 70% of all depressed patients report of difficulties in either the initiation or in maintenance of sleep [64–67]. Closely monitored biometric studies show that hypersomnia, or excessively long sleep episodes, are coupled with daytime sleepiness and frequent napping in 10–40% of patients with diagnosed mood disorders [68,69]. Indeed, alterations in sleep patterns are one of the key diagnostic criteria of depressive disorders. Conversely, depression may be observed as an effect of sleep disorders, showing reciprocity in the relationship between sleep and mood.

7. A role for adenosine and astrocytes in depression

Major depressive disorder (MDD) strikes one in 10 Americans in their lifetime. The effects of mood disorders such as MDD and bipolar disorder on individuals and society rank among the most disabling and costly of all medical illnesses. Numerous antidepressant pharmacotherapies are available in clinical practice, yet many patients undergo trial and error with multiple medications before achieving relief of symptoms. In addition, these pharmacological treatments take weeks to achieve their full efficacy, limiting their application to suicidal patients where rapid relief is necessary. These factors create a tremendous need to improve current treatment options for patients suffering from depression. Interestingly, a clinically employed non-pharmacological intervention that rapidly alleviates symptoms of depression is one or more nights of total sleep deprivation [70]. Sleep deprivation therapy is effective in approximately 60–70% of patients with depression [71,72].

The reduction in depressive symptoms observed following sleep deprivation correlates with the changes that can be seen in the slow wave activity (SWA) on baseline sleep. Changes in the amount of the rebound in right frontal all-night SWA during recovery sleep have also been shown to be significant [73,74]. MDD has been shown to change the sleep homeostat as measured by auditory evoked potential changes causing consequential changes in SWA. Finally, selectively sleep depriving subjects by only interrupting slow wave sleep is still an effective antidepressant treatment in patients with major depression [73,74].

Because the effects of sleep deprivation on depression are not long lasting, sleep deprivation is not always used clinically. However, if the mechanism mediating this action were identified it might be possible to develop therapeutics that target this pathway as a new treatment for certain forms of depression.

Signalling pathways involving adenosine have been linked to depression; however, controversy and inconsistencies exist as to whether adenosine (and its agonists) act in an antidepressant [75] or a depressant (El [76]) manner. A role for adenosine in depression is supported by the observation that 12 h of sleep deprivation elevates adenosine levels in rodent frontal cortex. Additionally, in patients with depression associated with sleep disorders, polymorphisms of the gene encoding the A1 receptor (ADORA-1) have been identified [77,78].

8. Astrocyte adenosine signalling regulates the antidepressant effects of sleep deprivation

We have recently shown that astrocytes are capable of modulating changes in non-rapid eye movement SWA in responses to sleep deprivation. In addition, these studies also showed that astrocytes regulate the amount of time that is spent in recovery sleep following sleep deprivation [3]. Expression of dnSNARE (dominant negative SNARE domain of the vesicle protein VAMP2) selectively within astrocytes reduces extracellular adenosine accumulation [3,79,80]. The dnSNARE mouse also shows reduced activation of A1 receptors. The lack of adenosine accumulation and reduced A1 receptor activation result in a reduction in sleep pressure and the electrophysiological changes associated with sleep deprivation [3].

Based on these findings, we postulated that astrocytes may also play a role in the beneficial effects of sleep deprivation in depressed patients. We demonstrate that sleep deprivation reduces depressive-like symptoms in mouse models of depression with similarity to human depression patients. Specifically, using 12 h of sleep deprivation we were able to show a significant reduction in time spent immobile in forced swim and tail suspension tests. Furthermore, we showed that this reduction in depressive-like symptoms is not observed in dnSNARE mice, A1 receptor knock-out mice or in mice treated with an adenosine receptor antagonist. These findings demonstrate that the anti-depressive effects of sleep deprivation require astrocytic SNARE-dependent signalling that is mediated through the A1 receptor.

9. A general role for astrocytic adenosine regulation in psychiatric disorders

Recent research strongly implicates astrocytes in modulating sleep [3,80,81]. Although very little is known about the direct mechanism, astrocytic adenosine has been shown to be a major player in sleep homeostasis. It is therefore quite logical that the astrocyte can be seen as playing a pivotal role in diseases that have sleep disruption as a hallmark. Many genes and their receptor products have been shown to be linked to adenosine dysregulation and psychiatric disorders (table 1). The next stage of important research will show what populations of cells in specific brain regions are responsible for specific sleep-related disorders.

Recent advancements in the glia field have shown these cells to play a role that is much greater than the 'glue that holds' grey matter together. Future research is already being directed towards manipulating specific astrocyte mechanisms relating to neurological disorders. It will be on the foundation of this basic research that some of tomorrow's clinical gains will be made.

Table 1. Single nucleotide polymorphisms identified in adenosine receptors, transporters and signalling molecules in depression and other neuropsychiatric disorders.

gene	single nucleotide polymorphism	disorder(s)	reference
ADORA1	rs17530497	depression with fatigue	[77]
	rs12026765	depression with fatigue	
ADORA2	1083C>T	panic disorder	[82]
	1976C>T	panic disorder	
	rs5751876	panic disorder	[83]
	rs5751862	anxiety-related personality	
	rs2298383	anxiety-related personality	
	rs3761422	anxiety-related personality	
	1976C>T	caffeine-induced anxiety	[84]
	2592C>T	caffeine-induced anxiety	
	rs2236624CC	autism spectrum	[85]
	rs3761422	autism spectrum	
	rs5751876	autism spectrum	
	rs35320474	autism spectrum	
ADA	rs6031682	depression	[77]
		depression with fatigue	
ADK	rs7924176	depression with fatigue	[77]
ENT1	rs693955	depression with morning awakening	[77]
	rs324148	depression with morning awakening	
	rs6905285	depression	
		depression with fatigue	
		depression with morning awakening	
ENT3	rs10999776	depression with morning awakening	[77]
	rs2066210	depression with fatigue	
	rs12256138	depression	
		depression with fatigue	
		depression with morning awakening	
	rs780659	depression	
		depression with morning awakening	
	rs780662	depression	
	rs12767108	depression	
		depression with morning awakening	
	rs2487067	depression	

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