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Chapter 10

ADENOSINE RECEPTOR MODULATION OF GABAERGIC TRANSMISSION

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ABSTRACT

Neurotransmission mediated by γ-aminobutyric acid (GABA) is well established, as GABA is the predominant inhibitory transmitter in the adult brain. The multiplicity of inhibitory neurons, differences between phasic and tonic GABAergic signaling, together with the need to discriminate between inhibition of excitatory neurons from inhibition of inhibitory ones, increases the complexity, therefore the interest, of the analysis of neuromodulation of inhibitory transmission. The neuromodulator adenosine, by operating high affinity adenosine receptors, has in addition a double potential: to restrain transmission via A1 receptor (A1R) and to facilitate it via A2A receptor (A2AR). One could therefore anticipate a precise location of these receptors in key points of the network to control the activity of GABAergic neurons.

The present review shall discuss how GABA is regulated by adenosine and how this affects several brain functions, namely hippocampal network stability, retina, cerebellum, basal ganglia functioning and its implications for fine control of movement and of drug addiction, brain areas involved in sleep control and in the control of the autonomic nervous system. This cross-talk between adenosine and GABA is part of brain homeostasis regulation and neuroprotection. Some implications for therapy are also discussed.

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INTRODUCTION

The predominant inhibitory neurotransmitter in the central nervous system is γ-aminobutyric acid (GABA). Therefore, the control of its activity is a key process not only for overall excitability adjustment, but also for the establishment of patterns of neuronal synchronization/desynchronization. This is crucial for most brain functions where a tight balance between excitation and inhibition is required to adjust network activity to homeostatic needs. Evidence for modulation of GABAergic transmission by adenosine is observed in several brain areas relevant for fine control of movement, drug addition, sleep or central command of autonomic nervous system functioning. At the forebrain, evidence to date point to a predominant action of adenosine on excitatory transmission, in particular in the action of the most abundantly expressed adenosine receptor, the A1R.

Indeed, up to recently A1R have been considered to be mostly devoid of influence upon GABAergic transmission in the hippocampus. We now know that this applies only to phasic GABAergic transmission. As we will detail below, adenosine exerts selected actions in hippocampal interneurons controlling their GABAergic inputs, which adds to its influence upon glutamatergic transmission, therefore allowing a fine control of network oscillations and synchronization of hippocampal output. The evidence for the modulatory action of adenosine upon GABAergic transmission in the other brain areas, as well as the corresponding functional implications, namely fine control of movement, drug addiction, sleep control and regulation of the autonomic nervous system, will also be reviewed.

Before that, we will summarize the main aspects of GABAergic transmission, so that the implications of its modulation by adenosine can be better perceived.

MAJOR CHARACTERISTICS OF GABAERGIC TRANSMISSION

GABA is the main inhibitory neurotransmitter in the adult brain, being primary released by around 20% of brain neurons [1, 2]. When first described in neurons, GABA was shown to produce inhibitory hyperpolarizing responses [3] that were blocked by bicuculline [4]. The fast signaling action of GABA, responsible for inhibitory post-synaptic potentials (IPSP) is mediated by the chloride (Cl^-) permeable ionotropic receptor, the $GABA_A$ receptor ($GABA_AR$) [5]. GABA also operates metabotropic receptors, the $GABA_B$ receptor ($GABA_BR$) [6, 7] coupled, through a G protein, to potassium (K^+) channels at the postsynaptic site, which leads to the late inhibitory postsynaptic potential characteristic of a slow GABA response [8]. $GABA_BR$ at presynaptic nerve terminals mediate inhibition of transmitter release [9]. The ionotropic, Cl^-permeable, GABA receptor at a subpopulation of retinal neurons has pharmacological properties distinct from the most abundant $GABA_ARs$, and was initially referred to as $GABA_C$ [10] but later included in the $GABA_AR$ class, on the recommendations of IUPHAR Nomenclature Committee [11].

The $GABA_ARs$ are heteromeric pentamers with the five subunits arranged around a central pore. Each subunit exists in different isoforms. Current evidence shows that most $GABA_AR$ subtypes are formed from two copies of a single α subunit isoform, two copies of a single β subunit isoform, and one copy of another subunit, such as γ, δ, ε, π or θ [12]. The physiological significance of the structural heterogeneity of $GABA_ARs$ may lie on the

provision of functional diversity such as channel kinetics, affinity for GABA, rate of desensitization and susceptibility for transient chemical modification (e.g., phosphorylation) [13]. In all cases, the net flow response that results from the increase in membrane permeability to Cl^- caused by $GABA_AR$ activation depends on the relative concentrations of Cl^- at each side of the membrane and on the membrane potential of the cell. In most mature neurons of the central nervous system the expression of the K^+/Cl^- co-transporter 2 (KCC2) [14, 15], which is a Cl^- extruder, will result in a Cl^- equilibrium potential that is more negative than the resting membrane potential of the neuron [16, 17]. Activation of $GABA_AR$ in mature neurons is thus usually "inhibitory" for two main reasons: (1) the increase in the permeability to Cl^- causes a general increase in membrane input conductance that shunts the ability of excitatory potentials to depolarize the membrane; (2) the Cl^--mediated hyperpolarization of the membrane sums to any depolarizing signal arriving to the neuron, reducing the probability of the cell to fire an action potential [18, 19]. In immature and developing neurons, however, the activation of $GABA_AR$ can lead to membrane depolarization and, in some cases, firing of action potential [20–23]. This results from a higher intracellular concentration of Cl^- due to early developmental expression of $Na^+/K^+/2Cl^-$ co-transporter 1 (NKCC1) [24] and lack of expression of KCC2 [17]. This intracellular accumulation of Cl^- in immature neurons determines a Cl^- equilibrium potential less negative than the resting membrane potential, leading to an outflow of Cl^-, therefore to depolarization upon activation of $GABA_ARs$. Even in mature neurons, neuronal activity, such as epileptiform discharges, can transiently change the reversal potential for GABA and turn $GABA_AR$-mediated currents into depolarizing [25–31].

Information carried out by activation of $GABA_ARs$ can be categorized in two main groups, phasic transmission and tonic transmission. Phasic GABAergic transmission allows a fast and precisely-timed communication between the GABAergic presynaptic terminal and the postsynaptic target. With the arrival of an action potential at the interneuron axonal terminal, a pool of GABA-containing vesicles is synchronously released to the synaptic cleft in a calcium-dependent manner. This will transiently increase local GABA concentration up to about 1.5 to 3.0 mM that lasts for less than 1 ms [32]. Released GABA is rapidly removed from the synapse either by high affinity GABA transporters in presynaptic nerve terminals and surrounding astrocytes or, in a lesser extent, by passive diffusion [33, 34]. $GABA_ARs$ clustered opposite to the releasing site are activated, producing an inhibitory postsynaptic current (IPSC) that in its minimal amplitude may represent one quantum, i.e., the amount of GABA present in one vesicle, which gives rise to a miniature IPSC (mIPSC) [32, 35, 36]. For a mIPSC to occur there is no need of the arrival of the action potential to the nerve terminal, since vesicles can be spontaneously released.

Actually, whenever aiming to quantify solely the release probability or quantal size, action potential generation in the network is blocked by adding a voltage-dependent channel blocker, frequently tetrodotoxin (TTX), to the extracellular solution. Besides the $GABA_ARs$ at the active zone of neurotransmitter release, there are also receptors adjacent to the synaptic bouton that can be recruited. Their activation may result from diffusion of GABA, which reaches $GABA_ARs$ located perisynaptically or located in the nearby synapses [37, 38]. This form of inhibition can also be considered phasic transmission since it is time-locked to presynaptic GABA release and only transiently activates $GABA_ARs$.

Tonic $GABA_AR$-mediated inhibition results from the continuous activation of $GABA_ARs$ by low concentrations of ambient GABA.

Receptors responsible for this form of transmission have an extra- or perisynaptic location, which are in an ideal position to sense ambient levels of GABA continuously present in the extracellular spacebut are less influenced by huge fluctuations of GABA concentrations that occur at the synaptic level [38].

Other important property of these $GABA_AR$s is their high affinity for GABA, conferring the ability to sense very low concentrations of ambient GABA that range from tens of nanomolar to a few micromolar [39-41]. There are also $GABA_AR$s that can be activated even in the absence of any ligand and contributing to the tonic current [42].

In addition, $GABA_AR$s mediating tonic currents are slow desensitizing receptors [43, 44]. The source of ambient GABA mediating tonic inhibition varies in different brain regions, cell types or even synaptic anatomy.

The non-vesicular sources include release from astrocytes [45-47], reversed transport of GABA by its transporter [40], non-vesicular GABA exocytosis [48] and channel-mediated GABA release from glia [49]. Experimentally, $GABA_AR$-mediated tonic transmission can be recorded by exogenously applying a $GABA_AR$ antagonist while monitoring the holding current required to voltage-clamp the cell at a given membrane potential [50].

There are clear physiological differences between phasic and tonic neurotransmission, albeit the fact that both control neuronal excitability. In the adult central nervous system, phasic inhibition is mainly involved in suppressing the activity of principal glutamatergic cells and preventing over-excitation of neurons.

Besides this classical role of synaptic GABAergic transmission, fast and precisely timed phasic responses mediated by GABA-releasing interneurons have other important and complex functions in neuronal communication. These include a key role in feedback and feedforward inhibition of principal cells with consequent synchronization of population activity and induction and/or maintenance of rhythmic network oscillations (e.g., gamma and theta frequency oscillations). Tonic transmission, on the other hand, acts on a much larger time window when compared to phasic responses.

A persistent increase in GABA input conductance in a particular neuron will significantly contribute to a phenomena called “shunting effect” [51], leading to a decrease in neuronal excitability [52–54]. The physiological significance of this is that the same excitatory input arriving to a neuron (e.g., glutamatergic input) will lead to a decrease in the output firing rate of the same neuron. Also, there will be a reduction in the duration of the excitatory potentials, narrowing the temporal fidelity of the excitatory input and decreasing the overall gain of the neuronal input-output relationship [54, 55].

Although tonic conductances can be considered as a constant and uninterrupted form of GABAergic transmission, changes in the concentration of ambient GABA or in the number and properties of extrasynaptic $GABA_AR$s contribute to changes in the magnitude of tonic inhibition, acting to control and fine-tune neuronal excitability [56]. Because tonic and phasic inhibition display distinct functional roles in GABA-mediated actions, selectively modulating these different forms of inhibition also affect the network excitability differently.

MAIN BRAIN AREAS AND FUNCTIONS WHERE ADENOSINE HAS BEEN SHOWN TO INFLUENCE GABAERGIC FUNCTION

Cerebral Cortex, Hippocampus and Amygdala

Early evidence for a relevant facilitatory action of A2AR upon GABAergic transmission was provided by John Phillis showing that the A2AR-mediated inhibitory action of GCS 21680 on spontaneous neuronal firing in vivo was prevented upon blockade of $GABA_A$Rs [57], suggesting that in the cerebral cortex A2AR actions on GABAergic transmission predominate over those on excitatory transmission. The same team has also reported that the selective activation of either A1R or A2AR results in an inhibition of ischemia-evoked GABA release in vivo [58]. A2AR-induced inhibition of GABA release from hippocampal slices under ischemic conditions have been also reported in a study using rather high concentrations of selective ligands [59]. Modifications of GABA release without experimental blockade of glutamatergic transmission are hard to interpret since GABA-releasing neurons in the forebrain are interneurons. Therefore, it is hard to distinguish between direct actions on GABAergic nerve terminals from disynaptic changes through modification of glutamatergic inputs to GABAergic neurons. By using isolated nerve terminals of the rat hippocampus it has been shown that a selective concentration of an A2AR agonist enhances depolarization-evoked GABA release, while selective concentrations of an A1R agonist were devoid of effect upon GABA release [60].

It is widely accepted that, in contrast with A2AR, A1R are mostly devoid of effect upon exocytotic GABA release or upon phasic GABAergic transmission at the hippocampus [60–65]. This lack of effect of A1R led to the idea that GABAergic transmission in the hippocampus is insensitive to A1R modulation; as we will detail in this section, there is now solid evidence that other components of GABAergic transmission are under A1R-mediated control. A first report on the absence of influence of A1R agonists (locally perfused in the hippocampus in vivo) upon veratridine- or hypoxia-induced release of GABA, quantified through microdialysis and HPLC, appeared more than 20 years ago [66]. In this study A1R agonists had a marked inhibitory action upon glutamate release. By using hippocampal slices, it was then reported that adenosine, while reducing the K^+-evoked release of glutamate and aspartate, did not affect the evoked release of GABA [61, 67]. Similar findings were reported by using an electrophysiological approach in cultured rat hippocampal explants [62], acute hippocampal slices [63] or cultured neurons [64]. All these studies showed that adenosine did not suppress fast IPSCs, but inhibited excitatory postsynaptic currents (EPSCs). Interestingly, one study noted that adenosine depressed late IPSPs evoked by direct activation of interneurons in the presence of glutamate receptor blockers [63]. Spontaneous quantal release of GABA, assessed by recording mISPCs in hippocampal slices in the presence of glutamate receptor antagonists and sodium channel blockade, is also not affected by A1R activation [65]. Furthermore, the failure to influence phasic GABAergic transmission applies either when recording from pyramidal neurons as well as while recording from interneurons [65].

In spite of not affecting GABA release from GABAergic nerve terminals at the hippocampus, A1R are able to modulate the action of other modulators of GABAergic transmission.

Thus CB1-mediated inhibitory actions upon GABA release are under control of adenosine A1R and this impacts upon forebrain-mediated behavioral actions of cannabinoids [68]. Thus, chronic caffeine intake, while upregulating A1R in the hippocampus, exacerbate memory deficits induced by acute intake of cannabinoids in the absence of caffeine [68]. Acute intake of caffeine or of an A1R antagonist together with cannabinoids also exacerbates memory impairment caused by cannabinoids, providing that the animals are naïve for caffeine [69]. In animals with A1R upregulated by chronic intake of caffeine, A1R blockade prevented cannabinoid-induced memory impairment [68]. These studies, in spite of highlighting a complex relationship between A1R and cannabinoid CB1 receptors (CB1R), that may involve both GABAergic and glutamagergic transmission, among others, provide clues from behavioraly relevant interaction between A1R and CB1R at hippocampal neurons.

Interestingly, in interneurons A1R actions are present in CB1R-positive interneurons, but are undetectable in CB1R-negative interneurons [65]. Modulatory actions of vasoactive intestinal peptide (VIP) upon GABA release are also under control of A1R, since endogenous adenosine removal with adenosine deaminase (ADA) or blockade of A1R prevents the facilitatory action of VIP upon GABA release, while activation of A1R in the presence of ADA rescues the effect of VIP [70]. A2AR activation is also required for the facilitatory action of VIP upon GABA release from hippocampal nerve terminals [70].

GABA release from immature hippocampal neurons seems sensitive to A1R activation, in contrast to what occurs in mature neuron under similar experimental conditions. Thus, in acutely isolated hippocampal neurons from P12-P15 rats, adenosine A1R activation causes presynaptic inhibition of GABAergic transmission, as assessed by the reduction of the frequency of mIPSCs recorded in the presence of glutamate receptor blockers and tetrodotoxin [71]. The same study reported absence of effect of A1R agonists in P30 neurons, therefore suggesting a developmental dependency of this inhibitory action of A1R [71]. In P5-7 neurons from cortical layer I, A1R blockade enhanced the amplitude of GABA-mediated fast synaptic responses, and decreased paired-pulse response ratio, suggesting a reduction of GABA release probability via presynaptic A1R activation by endogenous adenosine [72]. Interestingly, this action is also absent at P1-2 neurons [72]. Being evident at P5-7, a developmental stage where GABAegic activity can depolarize pyramidal neurons and influence glutamatergic synaptogenesis in the lower cortical layers, A1R may mediate developmental downregulation of the GABAergic excitatory drive when glutamatergic synapses are formed [72]. Delayed migration and insertion of GABAergic neurons into the hippocampal circuitry during the first postnatal week in offspring has been reported to occur when dams are treated with an A2AR antagonist or caffeine [73]. This was associated with increased neuronal network excitability and increased susceptibility to seizures, loss of hippocampal GABAergic neurons, and mild cognitive deficits, suggesting embryonic exposure to caffeine may have adverse consequences upon neuronal maturation [73].

The main output from the entorhinal cortex to the hippocampus is provided by stellate neurons. Interestingly A1R activation inhibits GABAergic inputs to stellate neurons, an action that involves presynaptic mechanisms as assessed by an inhibition of the frequency of mIPSCs [74]. Thus, it appears that while phasic GABAergic transmission within the hippocampus is unaffected by A1R activation [62–65], phasic GABAergic transmission to neurons that project into the hippocampus can be affected by A1R activation [74]. Neuronal responsiveness in the amygdala is largely controlled by inhibitory processes, which are also presynaptically downregulated by adenosine A1R activation [75].

While essentially not directly affecting phasic GABAergic transmission in mature neurons at the hippocampus, adenosine A1R at this brain area selectively modulate tonic inhibition mediated by activation of extrasynaptic $GABA_A$Rs [65]. The A1R-mediated modulation of tonic GABAergic currents is consistent in CA1 pyramidal cells, but present only in a specific population of postsynaptic CA1 GABAergic inhibitory interneurons, the CB1/CCK expressing interneurons [65]. Accordingly, sustained A1R activity results in a decreased expression of $GABA_A$R δ-subunit, a key component of extrasynaptic receptors mediating tonic GABAergic currents [65].

Since tonic GABAergic inhibition is more pronounced in interneurons than in pyramidal cells, the reduction in the disinhibition of interneurons caused by A1R-mediated suppression of tonic GABAergic transmission may increase inhibitory GABAergic output to the hippocampal principal cell population. The selective influence of A1R upon a specific interneuron population, the CB1/CCK expressing interneurons that are involved in synchronous network oscillations [76], may confer to adenosine an important modulatory action on hippocampal network oscillations that are the critical bases for hippocampal-dependent behavior and cognitive processes. A selective action upon tonic inhibition without affecting phasic inhibition may also confer to adenosine A1R a control of neuronal gain without disrupting fidelity of synaptic GABAergic inhibition. Such an action may prove relevant in the context of the anticonvulsant action of adenosine.

In contrast with A1R which, as mentioned above, inhibit tonic inhibitory GABAergic input to a subpopulation GABAergic interneurons in the hippocampus, the CB1R-positive neurons [65], A2ARs located in the nerve terminals of another subpopulation of interneurons (the parvalbumin-positive neurons, responsible for network synchronization) enhance phasic GABAergic inputs into other GABAergic interneurons, leading to disinhibition of pyramidal cells [77]. Interestingly, A2AR do not affect GABAergic inputs to excitatory neurons, as they also do not influence glutamatergic inputs to GABAergic neurons [77], but as expected A2AR enhance glutamatergic inputs to glutamatergic neurons [77]. These highly selective synapse- and cell type specific influence of adenosine A2AR, revealed by optogenetic stimulation of different interneuron subtypes combined with electrical stimulation of glutamatergic synapses, suggest an influence of A2AR upon synchronous pyramidal cell firing in hyperexcitable conditions. Accordingly, an A2AR antagonist was shown to robustly suppress spontaneous interictal like events in an ex vivo model of epilepsy [77].

Ischemia induces adaptive actions upon synaptic transmission and these can be influenced by adenosine that, as very well known, is released by neuronal cells under conditions of low oxygen or glucose supply [78–82]. Adenosine through A1R activation contributes to a post-synaptic enhancement of inhibitory synaptic transmission in CA1 neurons that occur for several hours after forebrain ischemia in vivo [83]. This may contribute to protect synapses, allowing functional recovery, similar to the A1R-mediated depression of glutamatergic synapses in an ex vivo model of hypoxia [84].

Influences of A1R upon the gating properties of $GABA_A$Rs also occur. This was first suggested through a rather indirect way, using a biochemical index that reveals changes in the function of postsynaptic $GABA_A$Rs, through modifications in the binding of butylbicyclophosphorothionate, known to bind to the ion recognition site of the $GABA_A$R as a function of its activity state; by using such approach it was suggested that A1R activation could reduce the function of the GABA-coupled chloride channel in several brain areas, including cortex and hippocampus [85].

The same indirect approaches also lead to the suggestion that caffeine administration in vivo significantly alters the Cl^- transport function of the $GABA_AR$ complex [86]. On the contrary, a positive interaction between adenosine and a $GABA_AR$ agonist, through a mechanism also involving chloride channels in hippocampal neurons, was also suggested to occur [87]. Control of $GABA_AR$ activity may be particularly relevant during seizures, where GABAergic responses transiently switch from hyperpolarizing to depolarizing, therefore excitatory and contributing to seizure progression. Under these conditions A1R-dependent activation of potassium channels increase membrane conductance and has a shunting effect on $GABA_AR$-mediated currents, which could limit seizure activity [88].

Other AR subtypes seem to play a role in the control of GABAergic currents during epilepsy, as antagonists of A2AR, A2BR and A3R (but not of A1R) in different epileptic tissues increase the stability of $GABA_AR$-mediated currents, assessed by a decreased run-down of the currents that occurs after repeated GABA applications [89, 90]. A reduction of the extracellular levels of adenosine through enhanced expression of adenosine kinase also leads to increased stability $GABA_AR$-mediated currents in pyramidal neurons of the hippocampus [91]. How this non-A1R mediated increase in the stability of GABAergic currents during seizures promotes excitatory GABAergic signaling and activity during seizures, is yet unknown. Seizure occurrence is in many cases effectively controlled by inhibitors of GABA transporters (GAT) which enhance extracellular GABA levels, and prolongs its inhibitory action, thus keeping neurons in a more hyperpolarized state. As such, modulation of GAT modulation hashas important implications for the control of epilepsy [34, 92]. GAT activity also determines the levels of ambient GABA available for extrasynaptic receptors and therefore for tonic inhibition. For experimental reasons, studies on modulation of tonic inhibition are performed under conditions of GAT blockade, which allows studying the activity of high affinity/slow desensitizing $GABA_ARs$ [65].

It is however also relevant to directly assess the influence upon GAT activity. Adenosine receptors are also able to modulate this aspect of GABAergic transmission. A2AR activation has been shown to enhance GABA transport into nerve endings [93], while the A1R is devoid of this function in nerve terminals. In astrocytes, A1R activation leads to an inhibition of GAT activity, while A2AR activation leads to its facilitation. This influence is observed either while evaluating GABA transport through GAT1 or GAT3, both present in astrocytes [94]. In addition, A2AR are required to gate the facilitatory action of brain-derived neurotrophic factor upon GAT1 mediated GABA transport into astrocytes [95].

Interestingly, there is a tight interaction between A1R and A2AR in the astrocytes to control GABA transport, so that blockade of one of the receptors prevents the activity of both receptors [94]. Actually, this regulation of GABA transport in astrocytes by adenosine occurs through A1R-A2AR heteromers (A1R-A2AR-A1R-A2AR tetramers) that signal via two different G proteins, G_s and $G_{i/0}$ and either enhances (A2AR) or inhibits (A1R) GABA uptake. Inhibition requires lower adenosine concentrations, but slight increases in concentration are enough to gate A2AR activation and to engage a completely opposite modulation of GABA uptake [94]. The levels of endogenous adenosine at the astrocytic vicinity may vary as a function of network excitability, since adenosine and ATP, a source of adenosine, are released in response to neuronal activity from the pre-synaptic, post-synaptic and astrocytic components [82, 96–98]. The presence or absence of microglia may also determine the levels of extracellular endogenous adenosine to control GABA transport into astrocytes [99].

One may then speculate that A1R-A2AR heteromers in astrocytes behave as adenosine sensors to fine-tune the synaptic levels of the inhibitory transmitter as a function of neuronal status. Thus, A1R-A2AR heteromers in astrocytes may behave as dual amplifiers, facilitating excitation at intense astrocytic to neuronal signalling and increasing inhibition at low neuronal firing rates. The main advantage of heteromerization of A1R and A2AR in astrocytes must be to avoid sudden transitions between inhibition and excitation.

Indeed, overstimulation of just one of the receptor leads to internalization of the whole functional unit [94] and probably avoids a sudden switch from excitation to inhibition or vice-versa as a consequence of desensitization of only one of the receptor subtypes.

In contrast to what occurs in the hippocampus [93, 94], in slices of the rat globus pallidus GAT1 mediated GABA transport is inhibited by adenosine A2AR activation, through a PKA-dependent mechanism [100]. A2AR-mediated facilitation of GAT1 activity in hippocampal nerve endings also involves a cyclic AMP/PKA-dependent mechanism [93], suggesting that similar mechanisms may have opposing actions upon GAT activity, depending upon the brain area involved. Indeed, GAT1 activity in slices from the globus pallidus is inhibited by substances which increase protein kinase A activity, such as forskolin and 8-bromo-cAMP [100], while GAT1 activity in hippocampal synaptosomes is enhanced by forskolin [93]. Differences resulting from the use of different preparations (slices vs isolated nerve endings) cannot be fully discarded, though the data is consistent with GAT1 modulation by A2AR activation in different preparations (isolated nerve endings vs cultured astrocytes) in the same brain area (forebrain).

Endogenous GABA might exert an inhibitory effect over adenosine A1R-mediated responses in the hippocampus [101], which may represent a physiologic regulatory mechanism between the two inhibitory mediators. Conversely, A1R activation may mask $GABA_A$R-mediated responses under certain conditions [102]. Thus, blockade of $GABA_A$Rs induces a potentiation of the inhibitory action of A1R upon synaptic transmission, an effect that involves NO acting through guanylyl cyclase [101].

Also, a GABAergic contribution to the depression of synaptic transmission caused by hypoxia, can only be revealed when A1R are blocked [102]. In conclusion, the data summarized above clearly indicates that adenosine acting on A1R and A2AR at pre-, post- and non-synaptic sites affects GABAergic transmission at the hippocampus.

Retina

Depolarization-evoked GABA release from retinal cells is not affected by A1R activation, though A1R present at the retina are able to mediate inhibition of acethylcholine release [103]. Therefore, the absence of modulation of phasic GABAergic transmission by A1R activationin retinal neurons is similar to observations in hippocampal neurons.

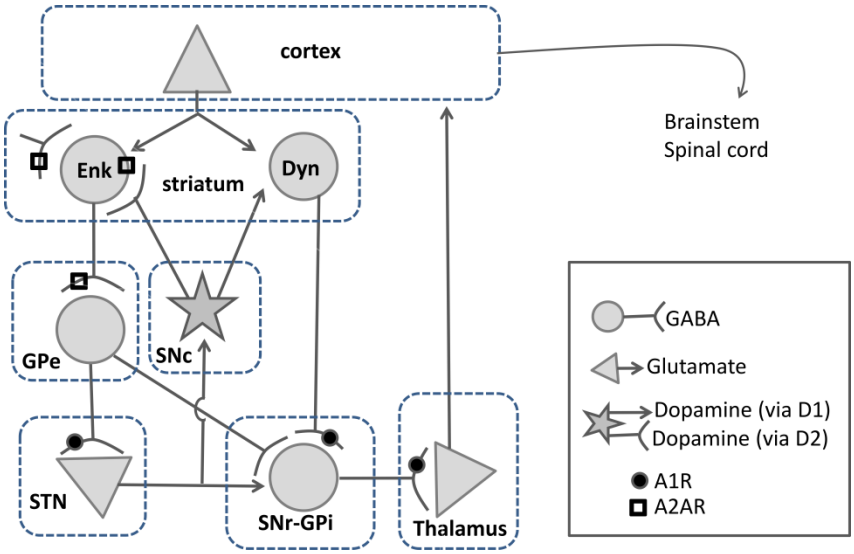
Retinal A1R, however, regulates GABA release mediated by reversal of the GABA transporters. In retinal explants of chick embryo, it was shown that caffeine and a selective A1R antagonist potentiates GAT1 mediated GABA release induced by activation of excitatory amino acid receptors [104], suggestive of tonic inhibition of GAT1 activity by A1Rs. Again, there is a similarity between the influence of adenosine A1R-mediated modulation of GAT activity in the retina and in forebrain areas since A1R activation leads to an inhibition of GAT activity in cultured astrocytes [104].

Cerebellum

Information on the actions of ARs at the cerebellum is scarce. It is known that A1R mediate presynaptic inhibition of GABAergic inputs to cerebellar granule cells [105]. Adenosine release in the rat cerebellum, on the other hand, seems to be under control of $GABA_BR$ activation [106]. Interestingly A1R activation at the cerebellum also inhibits adenosine release [106], suggesting that adenosine receptors can control the release of their ligand. A similar process also occurs at the hippocampus [80] and at chromaffin cells [107], though in these cases, control of adenosine transporters is mediated by A2ARs.

Basal Ganglia and Fine Control of Movement

It is well established that the predominant adenosine receptor at the basal ganglia is the excitatory adenosine A2AR, with a main (but not exclusive) location in the medium spiny GABAergic neurons, where A2AR are located postsynaptically to control afferent dopaminergic transmission (Figure 1). The selective expression of adenosine A2ARs in striatal enkephalin-expressing GABAergic medium spiny neurons, but not in striatal somatostatin-expressing GABAergic cells, suggests that adenosine may play a selective modulatory role in the activity of GABA/enkephalin striatopallidal neurones [108].



Enk: encephalin positive GABAergic neuron; Dyn: dynorphin positive GABAergic neuron; SNr-Gpi: substancia nigra pars compacta-globus pallidus pars interna complex; SNc: substantia nigra pars compacta; Gpe: globus pallidus pars externa; STN: subthalamic nucleus.

Figure 1. Schematic location of adenosine A1R and A2AR on GABAergic neurons to control basal ganglia circuitry. Presynaptic A2ARs at the basal ganglia tend to inhibit GABA release, whereas postsynaptic A2AR in enkephalin containing neurons inhibit D2R-mediated inhibitory signaling; presynaptic A1R inhibit GABA release (See text for references). The presence of A1R or A2AR in glutamatergic or dopaminergic neurons is not represented, but they are known to inhibit (A1R) or facilitate (A2AR) the release of glutamate at several synapses [112]. A2AR may also be present at dopaminergic nerve terminals to control the action of another neuromodulator (see text for details).

By studying the effect of intrastriatal infusion of adenosine and dopamine receptor ligands and evaluating the release of GABA from the terminals of the striopallidal neuron in awake, freely moving rats, it was also recognized that the GABAergic striopallidal neuron, which is a key component of the indirect striatal efferent pathway, is a main locus for A2AR and dopamine D2 receptor (A2AR-D2R) interactions in the brain and a main target for the actions of A2AR in the basal ganglia [109]. In this study it was shown that while intrastriatal infusion of A2AR agonists did not affect GABA release in the ipsilateral globus pallidus, they markedly antagonized the inhibitory action of D2R agonists upon GABA release in the same brain area [109]. It is now fully established that A2AR in the basal ganglia mostly operate in the indirect pathway, by modifying the D2R-mediated dopaminergic input to GABAergic striatal neurons that project to the globus pallidus pars externa. The implication of this action for fine control of movement has been the focus of several reviews [110, 111].

Evidence that striatal A2AR activation increases spontaneous GABA outflow through a mechanism that relates to the ability of A2AR to counteract the inhibitory influence of D2R-mediated responses has been provided in an early study using a microdialysis approach [113]. It was also that A2AR activation enhances GABA release in slices of striatum containing globus pallidus, and that the negative control exerted by D2R activation is counteracted by A2AR [114]. Using an acute model of Parkinson's disease (PD), it was shown that microinjection of an A2AR agonist into the striatum of non-lesioned animals increased GABA concentrations in the globus pallidus, whereas the injection of an A2AR antagonist reverses the increase in GABA release caused by 6-hydroxydopamine, an action related to the antiparkinsonian effects of A2AR antagonists [115]. Soon after, it was shown that enhancement of striatopallidal GABAergic transmission caused by metabotropic mGLuR5 receptor activation in either the ventral or dorsal striatum is potentiated by co-activation of A2AR, consistent with the idea of a synergistic interaction between mGluR5 and A2AR to facilitate GABAergic transmission in the basal ganglia [116].

As mentioned above, A2AR are predominantly but not exclusively expressed postsynaptically in striatal GABAergic neurons. Ultrastructural localization of adenosine A2AR early suggested multiple cellular sites for modulation of GABAergic neurons in rat striatum [117]. The presence of A2AR in nerve terminals of GABAergic interneurons projecting to the striatopallidal GABAergic neurons has been suggested on the grounds that A2AR activation leads to a pre-synaptic inhibition of inhibitory GABAergic currents at the enkephalin-containing GABAergic medium spiny neurons of the striatum [118, 119]. Pre-synaptic inhibitory actions of A2ARs upon GABA release were also found while using isolated nerve terminals from the striatum [120, 121]. Interestingly, this A2AR-mediated action is apparently not mediated by increases in intracellular cAMP [120]. Inhibition of GABAergic inputs to the striatum will also enhance the activity of striatum-pallidal neurons, thereby decreasing inhibition of subtalamic neurons by GABAergic neurons originated in the globus pallidus. Presynaptic facilitatory actions of A2AR at terminals of striatopallidal medium spiny neurons of the globus pallidus [122–124], will also add to this overall A2AR-mediated disinhibition of glutamatergic subthalamic nucleus activity. This would enhance inhibition of the thalamus by substantia nigra pars reticulate/globus palludus pars interna (SNr/GPi), an output structure of the whole basal ganglia network that projects to the thalamus through inhibitory GABAergic neurons. Oral administration of an A2AR-selective antagonist to an animal model of PD caused a marked and sustained increase of GABA and glutamate levels in the SNr. Selective lesion studies indicate that changes in neurotransmitter

release in the SNr brought about by A2AR in the basal ganglia is indeed mostly dependent on their ability to modulate the activity of striatopallidal medium spiny enkephalin-positive neurons that belong to the indirect basal ganglia pathway to control thalamic activity [125]. This facilitatory action of A2AR upon GABA release in the globus pallidus depends, at least in part, from the co-activation of dopamine D2R [126, 127] and is counteracted by co-activation of histamine H3R present in GABAergic terminals of the globus pallidus [128].

The presence of adenosine A2AR in dopaminergic nerve ending was suggested on the grounds of the facilitatory action of glial-derived neurotrophic factor (GDNF) upon dopamine release from isolated striatal nerve endings [129]. GDNF receptors are present in GABAergic nerve terminals of the striatum, though with lower expression than in glutamatergic and dopaminergic nerve terminals [130].

Interestingly the facilitatory action that A2AR exert over GDNF upon dopamine release seems to be under control of GABAergic neurons since in systems where neuronal connections are more preserved, as in striatal slices, the cross-talk between adenosine A2AR and GDNF is markedly affected by drugs that influence GABAergic transmission [129].

It has been shown that adenosine A2AR activation reduce GAT1 mediated GABA uptake in slices of the rat globus pallidus [100]. Interestingly, this is in contrast with what occurs in the forebrain, where A2AR activation facilitates GAT1 mediated GABA uptake into nerve endings [93] as well as GAT1 and GAT3 mediated GABA transport into astrocytes [94]. Since inhibition of GABA transport contributes to an increase in extracellular levels of GABAand enhanced inhibition, one might speculate that at the globus pallidus the action of A2AR upon GAT activity may enhance inhibitory tone of globus pallidus neurons, thereby reinforcing the disinhibition of subthalamic excitatory neurons.

Dopamine D1 receptor (D1R) mediated enhancement of the evoked release of GABA from SNr slices is inhibited by tonic A1R activation [131], suggestive of an A1R/D1R interaction to control the GABAergic inputs to SNr/GPi neurons.

Through this mechanism A1R activation may lead to the disinhibition of SNr/GPi neurons, hence, enhanced thalamic inhibition. Similarly, in ventral pallidum and nucleus accumbens, brain areas belonging to reward circuits and in addictive behavior, stimulation of D1R facilitates GABA release, an action attenuated by concurrent activation of A1R [132]. The facilitation of GABA release by D1Rs localized in the entopeduncular nucleus is also inhibited by A1R [133].

At the level of the thalamus, adenosine, through presynaptic A1Rs, strongly suppresses monosynaptic inhibitory currents both in relay cells of the thalamic ventrobasal complex and in inhibitory neurons of the nucleus reticularis thalami. The ability to presynaptically down-regulate inhibitory postsynaptic responses in thalamus and influence excitatory transmission accounts for the robust antioscillatory effects of A1R activation [134].

Whole-cell current recordings in SNr neurons which receive input from dynorphin containing neurons that, in turn, receive dopaminergic inputs through D1R (direct pathway), revealed that A1R, through a presynaptic mechanism, decreases GABAergic inputs to SNr neurons [135]. This suggests an action of the A1R in the nerve endings of the dynorphin-positive neurons. Consistent with these findings, it was recently shown that adenosine, acting on A1R, blunts D1R enhancement of spontaneous GABAergic synaptic activity in SNr neurons [136]. A decreased inhibition of SNr neurons and consequent increased output from these neurons is known to contribute to the rigidity and bradykinesia in PD [110].

Remarkably, A1R administration in vivo in combination with L-DOPA, reduced the development of abnormal involuntary movements, highlighting the potential benefit of A1R agonists for the treatment of L-DOPA-induced dyskinesia and hyperkinetic disorders [136].

A1Rs also seem to be present in the indirect pathway, as electrophysiological recordings from subthalamic nucleus neurons revealed an A1R-mediated presynaptic inhibition of both GABAergic and glutamatergic inputs to those neurons [137].

Endogenous adenosine, acting on presynaptic A1R, is responsible for early presynaptic depression of IPSCs caused by oxygen/glucose deprivation in striatal slices [138], in clear contrast with the hippocampus where monosynaptic IPSCs are more resistant to hypoxiaand to synaptic modulation by adenosine A1Rs [139].

At the hippocampus, adenosine A1R-mediated inhibition of excitatory synaptic transmission during hypoxia favors recovery after reoxygenation [84]. Whether the early depression of GABA-mediated synaptic inhibition exerted by A1R at the striatum may play a role in the development of ischemic neuronal injury is unknown.

BRAIN AREAS RELATED TO DRUG ADDICTION

The dopaminergic projections from the ventral tegmental area (VTA) into the nucleus accumbens, major players in the reward circuit, are under control of GABAergic neurons that make synapse either at the level of the VTA or at the level of the nucleus accumbens. A1R inhibit GABAergic inputs to nucleus accumbens neurons [140]. Furthermore, the facilitatory action of dopamine D1R upon GABA release at the nucleus accumbens and ventral pallidum is attenuated by concurrent activation of adenosine A1R [132]. In contrast, A2AR may facilitate GABA release in these areas since an A2AR antagonist, KW6002, decreases GABA release while increasing dopamine release from the nucleus accumbens [141].

Patch clamp recordings of GABAergic currents in dopaminergic neurons of the VTA provided evidence that adenosine, through presynaptic adenosine A1R, decreases the release of GABA and attenuated $GABA_BR$-mediated inhibition of dopaminergic neurons [142], therefore providing their disinhibition. Relevant interactions between adenosine A1R and type 2 receptors of corticotrophin releasing factor (CRF-R2) at GABAergic nerve terminals have been recently shown in a cocaine addiction and reinstatement model, which helps to understand the cellular basis for relapse-mediated changes in the activity of VTA dopaminergic neurons [143]. Thus, evidence has been obtained consistent with the interpretation that reinstatement leads to a CRF-R2 dependent enhanced activity of adenosine A1R on VTA GABAergic nerve terminals. This causes a decreasein GABAergic tone and a lower activation of $GABA_BRs$ at the glutamatergic nerve terminalsand increased glutamatergic inputs to VTA dopaminergic neurons [143]. While A1R in VTA may have a facilitatory influence upon reinstatement of cocaine abuse, A2AR in the nucleus accumbens seems to reduce reward associated with cocaine self-administration, an action that also involves changes in the GABAergic tone. This has been suggested based on findings that activation of A2AR attenuates the increase in dopamine levels in the nucleus accumbens caused by cocaine self-administration, while simultaneously enhance GABA levels in the same brain area [144]. This action may be related to A2AR-induced attenuation of inhibitory actions of D2R upon GABAergic neurons in the accumbens, therefore leading to reduced

cocaine-induced reward [144]. Also indicative of the key role of adenosine/GABA interplay during different steps of addictive behavior is a study showing that hyperexcitability during morphine withdrawal is retarded with a concomitant increase in endogenous A1R activation by adenosine [145]. Thus, in chronically morphine treated mice both the rate of mIPSCs and the amplitude of evoked IPSCs during naloxone-precipitated withdrawal was profoundly enhanced in the presence of an adenosine A1R antagonist [145].

BRAIN AREAS INVOLVED IN SLEEP CONTROL

Both A1R and A2AR influence sleep control, by acting in several areas and by operating different mechanisms [112, 146–148]. These actions that involve GABAergic transmission in brain areas more directly related to sleep regulation are summarized below.

Adenosine acts presynaptically to decrease the probability of spontaneous GABA inputs onto tuberomammillary nucleus (TMN) neurons [149], which have important roles in the homeostatic control of sleep. GABAergic innervation from the ventrolateral preoptic nucleus (VLPO) to the TMN also plays pivotal roles in the regulation of sleep-wakefulness [150]. Microinjections of adenosine into the medial preoptic area induces sleep and the early finding that this action is prevented by the non-competitive $GABA_AR$ antagonist flumazenil constituted an initial evidence that the hypnotic properties of adenosine could be mediated by a direct or indirect action on the $GABA_A$-benzodiazepine receptor complex [151]. Activation of presynaptic A1R decreases spontaneous GABAergic transmission onto TMN neurons via the modulation of G-protein coupled inward rectifying potassium (GIRK) channels as well as the adenylate cyclase/cyclic AMP/protein kinase A signal transduction pathway. This adenosine A1R-mediated modulation of GABAergic transmission onto TMN neurons may play an important role in the fine modulation of the excitability of TMN histaminergic neurons as well as the regulation of sleep-wakefulness [149]. Furthermore, adenosine, via the activation of A2AR, stimulates a subpopulation of VLPO GABAergic neurons [152].

GABAergic inputs to hypocretin neurons in the lateral hypothalamus, a wakefulness promoting center, are presynaptically inhibited by A1R located in GABAergic nerve terminals, a process that may contribute to fine regulation of the excitability of these neurons as well as eventually to modulate the sleep–wakefulness cycle [153]. Disinhibition of sleep promotion neurons of the ventrolateral preoptic area of the hypothalamus through inhibitory presynaptic A1R located in GABAergic neurons has also been reported [154].

In freely moving rats, application of an A2AR agonist, CGS 21680, to the subarachnoid space underlying the rostral basal forebrain significantly promoted sleep and inhibited histamine release in the frontal cortex [155]. While inhibiting histamine release in both the frontal cortex and medial pre-optic area, GCS 21680 increased GABA release specifically in the histaminergic TNM neurons but not in the frontal cortex [155].

Moreover, the CGS 21680-induced inhibition of histamine release was antagonized by perfusion of the TMN with a GABAAR antagonist, picrotoxin [155]. Altogether, these data suggest that the A2AR activation can induce sleep by inhibiting the histaminergic system through an increase in GABA release in the TMN [155].

In cultured neurons of the arcuate nucleus and of the suprachiasmatic nucleus of the hypothalamus, the control center of mammalian circadian rhythms, adenosine inhibits synaptic GABA release, an action that may involve not only A1R but also A2AR [156].

CIRCUITS INVOLVED IN PAIN CONTROL

The existence of an interaction between adenosine-mediated mechanisms and GABAergic mechanisms to control pain was initially suggested on the grounds that a $GABA_AR$ and $GABA_BR$ antagonists prevented antinociceptive action of A1R agonists, and that a $GABA_BR$ agonist potentiate the antinociceptive action of a A1R agonist [157].

Adenosine suppresses GABAergic and glycinergic transmission in substantia gelatinosa neurons by activating presynaptic A1R, an action that may contribute to the modulation of pain transmission [158]. Also in the spinal cord, but at the sacral dorsal commissural nucleus, an area involved in central processing of pelvic visceral information, adenosine, by operating post-synaptic A1R, suppresses GABA-induced Cl^- currents, suggestive of an involvement of adenosine in visceral pain control [159]. This action of adenosine involves a Ca^{2+}-insensitive protein kinase C-dependent mechanism [159]. Similar post-synaptic actions of A1R have been detected in the superficial laminae (laminae I and II) of the rat spinal dorsal horn [160]. In addition, convergent and activity-dependent inhibition of GABA release by A1 and GABAB autoreceptors have been shown to occur in dorsal horn neurons of the spinal cord, suggestive of a modulation of the integrative properties of these neurons under physiological conditions and/or during the development of pathological pain states [161].

In isolated rat dorsal root ganglion neurons adenosine A1R inhibit inhibitory currents mediated by $GABA_ARs$ by shifting the GABA concentration-response curve downward without affecting the apparent affinity of GABA [162]. This inhibitory effect of adenosine A1R upon GABAergic currents requires protein kinase C activity and is mimicked by phorbol esters, suggesting involvement of the phospholipase C/protein kinase C transducing pathway [162]. Since GABA is an important neurotransmitter in the generation of presynaptic inhibition in the dorsal horn to modulate sensory inputs such as pain, this inhibitory action of adenosine upon an inhibitory neurotransmitter may impact upon pain processing and synaptic plasticity during overstimulation, namely in wind up phenomena observed in certain spinal sensory pathways [162].

BRAIN AREAS INVOLVED IN THE CONTROL OF AUTONOMIC NERVOUS SYSTEM

Magnocellular neurosecretory cells synthesize vasopressin, known to play a key role in body fluid homeostasis. The cell bodies of magnocellular cells are located at the hypothalamus and project their axons to the neurohypophysis, controlling hormone release. Thus, GABAergic inputs to the cell body of magnocellular neurons impact hormone secretion and fluid homeostasis. Adenosine, via A1R, inhibits synaptic GABAergic inputs to the cell body of magnocellular cells located at supraoptic nucleus of the hypothalamus [163].

The pre-sympathetic neurons in the hypothalamic paraventricular nucleus play an important role in regulating arterial blood pressure and sympathetic outflow through projections to the spinal cord and brainstem. Adenosine A1R expressed on spinally projecting hypothalamic paraventricular nucleus neurons hyperpolarize these neurons and decrease their firing activity [164]. In addition, the GABAergic and glutamatergic inputs to these neurons are also inhibited by adenosine, indicative of the presence of these receptors in nerve terminals that synapse with hypothalamic paraventricular neurons [164]. A presynaptic inhibitory action of adenosine A1R upon spontaneous and evoked GABAergic inputs to neurons of the paraventricular nucleus, as assessed by a decrease in the frequency of mIPSC and an increase paired-pulse ratio of evoked IPSCs, was also reported [165]. These data reinforce previous evidence that adenosine, through control of GABAergic transmission, acts as an important neuromodulator of the central nervous system mediated cardiovascular regulation [166]. Adenosine A1R have been shown to presynaptically inhibit GABAergic inputs to the midbrain periaqueductal grey neurons, which also play a key role in the cardiovascular responses, in particular those related to stress or pain [167].

GABA and adenosine contribute to respiratory inhibition in early postnatal life. Thus, during early development, adenosine contributes to the occurrence of respiratory depression and recurrent apneas, an action that results from an interaction with GABAergic circuits since it is abolished upon blockade of GABAergic transmission [168]. A2AR mRNAs was detected in GABAergic neurons of brainstem areas involved in the central control of respiration, including bulbospinal GABAergic neurons projecting to the phrenic motor nuclei [169], reinforcing the idea that adenosine A2AR can induce respiratory depression through a facilitatory action over GABAergic neurons. In infant (14-16 days after birth), but not in adult rats, the intracerebroventricular injection of an A2AR agonist decreases the respiratory drive, an action prevented by prior GABAAR blockade, also suggestive of an action mediated via GABAergic inputs to the inspiratory timing neural circuitry [170]. A2AR-mediated amplification of GABAergic mechanisms in the nucleus tractus solitarius with implications for reflex respiratory control has also been demonstrated [171]. Interestingly, adenosine, through excitatory A2AR and A2BR located at the carotid body, mediate an increase in carotid sinus nerve firing frequency and an increase in the respiratory drive [172–175], but any relationship between these facilitatory actions and modulation of GABAergic activity within the carotid body neurons is as yet unknown.

CONCLUSION

As pointed out by Sebastião and Ribeiro [112], synchronization or desynchronization of neurons is essential for most brain functions. Dysfunctions of desynchronization, resulting in hypersynchronism at the temporal lobe, trigger epileptic seizures, and adenosine, as GABA, are known as antiepileptic agents. Indeed, the homeostasis of the brain depends primarily on regulatory molecules, such as GABA and adenosine, which modulate neuronal excitability and control the predominance of the activity at certain brain areas over others. Classical examples are brain oscillations put in evidence while coding specific information, setting and modulating brain attentional states, and assuring the communication between neuronal populations, which constitutes the material core of cognitive functions [176].

Evidence summarized in this review highlights the multiple points where adenosine can act at the tripartite synapse to control different aspects of GABAergic transmission. A role of adenosine upon tonic GABAergic inhibition, exerted either by modulation of GABA transporters at nerve endings and astrocytes, or by modulating high affinity/slow desensitizing $GABA_A$Rs, was demonstrated in detail in the hippocampus, basal ganglia and retina. This influence upon tonic inhibition, together with synapse specific actions upon phasic inhibition, will certainly contribute to adjust network activity in different brain areas. Such a function could underlie the adenosine 'destiny' - the homeostatic control of brain function.

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