

Adenosine A₁ receptor: Functional receptor-receptor interactions in the brain

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Abstract Over the past decade, many lines of investigation have shown that receptor-mediated signaling exhibits greater diversity than previously appreciated. Signal diversity arises from numerous factors, which include the formation of receptor dimers and interplay between different receptors. Using adenosine A₁ receptors as a paradigm of G protein-coupled receptors, this review focuses on how receptor-receptor interactions may contribute to regulation of the synaptic transmission within the central nervous system. The interactions with metabotropic dopamine, adenosine A_{2A}, A₃, neuropeptide Y, and purinergic P2Y₁ receptors will be described in the first part. The second part deals with interactions between A₁Rs and ionotropic receptors, especially GABA_A, NMDA, and P2X receptors as well as ATP-sensitive K⁺ channels. Finally, the review will discuss new approaches towards treating neurological disorders.

Keywords Adenosine · G protein-coupled receptors · Receptor interactions · Neurotransmission · Adenosine receptors · Ionotropic receptors

Abbreviations

A₁R adenosine A₁ receptor
A_{2A}R adenosine A_{2A} receptor
A_{2B}R adenosine A_{2B} receptor
A₃R adenosine A₃ receptor
2-CA 2-chloro-adenosine

CHA *N*⁶-cyclohexyladenosine
CI-IB- *N*⁶-(3-iodobenzyl)adenosine-5'-
MECA *N*-methyluronamide
CPA *N*⁶ cyclopentyladenosine
8-CPT 8-cyclopentyl-1,3-dimethyl-xanthine
D₁R dopamine D₁ receptor
D₂R dopamine D₂ receptor
DIDS chloride channel blocker
DPCPX 1,3-dipropyl-8-cyclopentylxanthine
EPSPs excitatory postsynaptic potentials
[F7,P34] Y₁R agonist
pNPY
GIRK₁₋₄ inward rectifying K⁺ channels
GPCR G protein-coupled receptor
GRAFS glutamate, rhodopsin, adhesion, fizzled/taste and secretin-like GPCRs
IP3 inositol 1,4,5-triphosphate
IPSPs inhibitory postsynaptic potentials
K_{ATP} ATP-sensitive K⁺ channels
LTP long-term potentiation
MPTP methylphenyltetrahydropyridine
MRS-1191 [3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(+/-)-dihydropyridine-3,5-dicarboxylate]
NHE1 Na⁺/H⁺ exchange
NPY neuropeptide Y
PLA phospholipase A
PLC phospholipase C
PSPs postsynaptic potentials
R-PIA (R)-N⁶-(2-phenylisopropyl)adenosine
SKF 38393 (+/-)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide
VDCC voltage-dependent Ca²⁺ channels
Y₁₋₅R neuropeptide Y₁₋₅ receptor

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Introduction

The vertebrate central nervous system (CNS) is characterized by a dynamic interplay between signal transduction molecules and their cellular targets. Modulation of synaptic transmission by metabotropic or ionotropic receptors is an important source of control and dynamical adjustment in synaptic activity.

Recent studies have provided new insights into the role of ligand-gated ion channels in modifying synaptic transmission. Along with a growing list of different types of pre- and postsynaptic ionotropic receptors and the cell types that express them, there have also been advances in characterizing the modulatory mechanisms of the receptors that link to receptor activation. This is important due to the convergence of data from biochemical, molecular, and electrophysiological studies, implicating ionotropic receptors in the effects of psychoactive and addictive drugs.

G protein-coupled receptors (GPCRs) make up the largest and most diverse family of membrane receptors in the human genome, relaying information on the presence of diverse extracellular stimuli to the cell interior. An estimated 1% of the mammalian genome encodes for GPCRs, and about 450 of the approximately 950 predicted human GPCRs are thought to be receptors for endogenous ligands [1]. The manipulation of transmembrane signaling by GPCRs may constitute the most important therapeutic target in medicine. Nearly 40% of all current therapeutic drugs target GPCRs [2].

All known GPCRs share a common architecture of seven membrane-spanning helices connected by intracellular and extracellular loops. Drugs acting on GPCRs have been classified as agonists, partial agonists, or antagonists based on a “two-state model of receptor function.” Since experimental evidence pointed out the impossibility of explaining the operation of GPCRs without considering dimers as the minimum structure for many GPCRs the “two-state dimer receptor model” was developed based on the communication between the two subunits of the receptor dimer [1, 3, 4]. This model is an extension of the “two-state model of receptor function” but considers dimeric structures able to bind one molecule to the orthosteric center in each monomer.

GPCR signaling is subject to extensive negative regulation through receptor desensitization, sequestration, and downregulation, termination of G protein activation by GTPase-activating proteins, and enzymatic degradation of second messengers. Additional protein-protein interactions positively modulate GPCR signaling by influencing ligand binding and specificity.

Multiprotein complexes mediate most cellular functions. In neurons, these complexes are directly involved in the neuronal transmission, which is responsible for learning, memory, and developments. The first publication in this

direction came from Hökfelt's group in 1983. The publication describes how substance P may modulate the high-affinity serotonin (5-HT) binding site in a spinal cord membrane preparation [5]. Over the past decade, the number and outcomes of interactions between receptors have increased continuously [6]. Recent studies have demonstrated close physical interactions where activation of one receptor affects the function of the other.

Adenosine is an endogenous purine nucleoside that has evolved to modulate many physiological processes. Extracellular adenosine mostly originates from release of intracellular adenosine and from release and extracellular breakdown of cAMP and ATP by ecto-5'-nucleotidase and phosphodiesterase [7]. Cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A_1 Rs, A_{2A} Rs, A_{2B} Rs, and A_3 Rs), all of which are seven-transmembrane-spanning GPCRs. Of the four known adenosine receptors, A_1 Rs and A_{2A} Rs are primarily responsible for the central effects of adenosine, especially in modulating synaptic transmission [8]. Adenosine can act on A_1 Rs to depress transmitter release and neuronal sensitivity to the transmitter [9, 10]. As a result, the A_1 Rs are important in the regulation of synaptic plasticity, playing a role in determining the amplitude of long-term potentiation or long-term depression [11].

There are numerous reviews that describe regulation of brain adenosine levels, adenosine receptors, their cellular and subcellular localization, signaling pathways, and function in the brain under physiological and pathophysiological conditions as well as selective receptor agonists and antagonists. Using A_1 Rs as a paradigm of GPCRs, this review focuses on how receptor-receptor interactions contribute to regulatory processes within the central nervous system.

Considering the various types of receptors, one may expect to find three principle paths of receptor interaction: (1) interactions between ionotropic receptors, (2) interactions between a metabotropic receptor and an ionotropic receptor, and (3) interactions between metabotropic receptors. The examples mentioned below stem from the second and third type of interaction. Interactions with metabotropic dopamine receptors as well as A_{2A} , A_3 , NPY, and $P2Y_1$ receptors will be described in the first part. The second part deals with interactions between A_1 Rs and ionotropic receptors, especially the $GABA_A$, NMDA, and $P2X$ receptors as well as ATP-sensitive K^+ channels. Finally, new approaches for neurological disorders will be discussed.

Functional interactions with metabotropic receptors

Two forms of GPCR classification exist. There is the historical division into three main families: (1) rhodopsin-like family which includes adenosine receptors, (2) secretin-like

family, and (3) metabotropic glutamate receptor-like family. The families share some basic similarities—the seven-transmembrane-spanning domains, intracellularly located C terminus, and extracellularly residing N terminus. Differences between the families arise in the length of the intracellular and extracellular termini and amino acid sequences, disulfide bridge linking, and conserved domains. Five different groups can be classified by applying phylogenetic analyses. The GRAFS system distinguishes between glutamate, rhodopsin, adhesion, fizzled/taste, and secretin-like GPCRs [12].

The agonist binding on the receptor results in coupling to heterotrimeric G proteins and regulates a variety of cell responses. In brief, an exchange of G protein-bound GDP to GTP occurs, and the heterotrimer dissociates into the α subunit and the $\beta\chi$ dimer. The resulting products activate or inhibit effectors independently from each other. Currently, 16 different genes encode G protein α subunits, five genes encode β subunits, while 14 genes encode χ subunits [13]. The α subunits can be categorized into four basic groups: the stimulatory $G_{\alpha s}$ family couples to adenylate cyclase and increases cAMP levels, whereas the inhibitory $G_{\alpha i/0}$ family acts in the opposite way. Moreover, the $G_{\alpha q/11}$ family activates the phospholipase C_{β} (PLC_{β}), and lastly, the $G_{\alpha 12/13}$ family which regulates Rho proteins. $G_{\beta\delta}$ dimers are capable of triggering effects on inward rectifier K^+ channels ($GIRK_{1-4}$), voltage-dependent Ca^{2+} channels (VDCC), and phospholipase A_2 (PLA_2), PLC_{β} , and the Na^+/H^+ exchanger (NHE1). Thus, it is not surprising that GPCRs are such interesting candidates in current drug research with their amazing potential in affecting signaling events. A single GPCR possesses the potential to activate more than just one signaling pathway [12]; for example, activation of A_1 Rs includes coupling to $G_{\alpha i/0}$ and increasing of IP₃ level [14, 15]. Furthermore, homodimerization and heterodimerization are common paths of interaction and have been described in A_1 Rs and the A_2 Rs several times [16–18]. In addition, functional interactions on the A_1 R without receptor assembling have already been revealed [19, 20], or are currently being elucidated. The next paragraphs will deal with a few selected examples of this limitlessly wide topic.

Relationship between A_1 Rs and A_3 Rs

The A_3 R was the latest receptor subtype of the adenosine receptor family to be identified [21], and its functional role is still controversially discussed. Several findings indicate neuroprotective as well as neurotoxic action depending on experimental approach [22–29]. A_3 Rs couple to inhibition of adenylyl cyclase as well as to activation of PLC, and to elevation of inositol triphosphate levels [30, 31]. Furthermore, an increase in intracellular Ca^{2+} levels due to release

from intracellular stores and Ca^{2+} influx has been described [32, 33]. One interesting example of A_3 Rs' functional role is their involvement in acute neurotoxic situations and interplay with A_1 Rs. Dunwiddie et al. [34] reported on the potential of A_3 Rs to modify responses via A_1 Rs in the hippocampus. The activation of hippocampal A_3 Rs induced a desensitization of A_1 Rs on combined superfusion of Cl-IB-MECA and adenosine. This phenomenon was thought to reduce the protective effects of endogenous adenosine caused by the lack of sensitivity of A_1 Rs. Further investigations on pyramidal cells of the rat cingulate cortex did not confirm Dunwiddie et al.'s assumption [35]. In this brain area, A_1 Rs and A_3 Rs did not show any interaction. The receptor subtypes were unable to affect each other. The discrepancy was taken to be a genetic phenomenon, such as alternative splicing of the rat A_3 R transcript causing distinguished pharmacological and functional properties in the brain. Furthermore, Hentschel et al. [36] demonstrated the involvement of A_3 Rs in inhibition of excitatory neurotransmission during hypoxic conditions, indicating a neuroprotective action of endogenously released adenosine on A_3 Rs in addition to A_1 Rs. Lastly, Lopes et al. [37] attempted to define the possible role of A_3 Rs in the rat hippocampus using experiments similar to those of Dunwiddie et al. in non-stressful and stressful situations, with particular attention to whether A_3 Rs control A_1 Rs. These data suggested that no interaction between the two receptor subtypes exist, but confirm that A_3 Rs do not affect synaptic transmission on superfusion with A_3 R agonist Cl-IB-MECA or A_3 R antagonist MRS 1191. The authors pointed out that Cl-IB-MECA binds to A_1 Rs even at low nanomolar concentrations. Thus, the existence of an interaction between A_1 Rs and A_3 Rs has to wait for reliable ligands.

Antagonistic interaction between A_1 Rs and A_{2A} Rs

A_{2A} Rs are widely distributed in the CNS, but local and subcellular differences in allocation exist. They show high levels in all subregions of the striatum and in the globus pallidus. A_{2A} Rs are also expressed in neurons in the neocortex and limbic cortex, but at a density a twentieth of that found in basal ganglia [38]. Colocalization of A_1 Rs and A_{2A} Rs was approved for glutamatergic nerve terminals in the hippocampus [39]. In the striatum, A_1 R/ A_{2A} R heteromers were found on synapses with spines of medium spiny neurons and integrated in the presynaptic membrane of glutamatergic terminals that represent the cortical-limbic-thalamic input [18]. A_1 Rs and A_{2A} Rs modulate excitatory synaptic transmission, albeit in an opposite manner. A_1 R activation inhibited glutamatergic synaptic transmission mainly through presynaptic inhibition of glutamate release, while A_{2A} Rs have been shown to facilitate glutamatergic synaptic transmission [40–42]. At first sight, stimulating

A₁Rs and inhibiting A_{2A}Rs may have a neuroprotective influence on the mature CNS. However, problems arise due to long-term desensitization of A₁Rs. A_{2A}Rs do not up-regulate after antagonist administration, but have a low abundance in hippocampal and cortical areas compared with A₁Rs [40, 43, 44]. A₁Rs and A_{2A}Rs cannot be regarded in isolation from one another since cross talk between the subtypes has been described several times [16, 17, 45–47]. A_{2A}R activation by agonists caused A₁R desensitization resulting in decreased binding affinity for CPA in the hippocampus in young adult rats. Controlling A₁Rs by A_{2A}Rs was mediated by protein kinase C in a cAMP-independent manner. A_{2A}R activation was seen to play a role in fine-tuning A₁Rs by attenuating the tonic effect of presynaptic A₁Rs located on glutamatergic nerve terminals [46, 47]. In the striatal system, A₁R/A_{2A}R heteromers became prominent to show an antagonistic reciprocal interaction [18]. As in the hippocampus, A_{2A}R stimulation decreased the affinity of A₁Rs for agonists. The A₁R/A_{2A}R heteromer allows adenosine to perform a detailed modulation of glutamate release [16, 48]. Regarding A₁Rs and A_{2A}Rs, basal conditions generate a low tone of endogenous adenosine and cause A₁R activation, in contrast to situations of increased adenosine where A_{2A}R activation becomes dominant. When adenosine concentrations rise, as during anoxia, likely also time appears to be important in regulating A_{2A} receptor activity, which means A_{2A} receptors are “active” under prolonged stimulation [49]. Finally, activation of the A₁R/A_{2A}R heteromer contributes to A_{2A}R signaling when adenosine levels are elevated and may provide a mechanism to facilitate plastic changes in the excitatory synapse [18].

Interactions between adenosine and dopaminergic system

Dopamine is an important transmitter in basal ganglia and is noted for influencing motor activity, playing an important role in Parkinson’s disease. Adenosine-dopamine interactions are complex and cannot be limited on functional considerations of A₁Rs. Intramembrane heteromeric receptor-receptor interactions and the involvement of A_{2A}Rs in influencing dopaminergic signaling have to be mentioned due to the implications in the treatment of Parkinson’s disease. Ginés et al. [50] described the formation of functionally interacting heteromeric complexes between dopamine D₁ receptors (D₁Rs) and A₁Rs in mouse fibroblast Ltk⁻ cells cotransfected with respective cDNAs. Coaggregation occurred when cells were pretreated with R-PIA as A₁R agonist, but was decreased by combined pretreatment with R-PIA and SKF-38393, a D₁R agonist. Furthermore, the D₁R agonist-induced cAMP accumulation was reduced by combined pretreatment of D₁R- and A₁R agonist, but remained unaffected when given alone, respectively. The results confirmed an antagonistic interaction between A₁Rs

and D₁Rs that had already been observed by Ferré et al. [51] in behavioral studies using reserpinized mice and rabbits. In vivo and in vitro data on adenosine-dopamine interactions were mostly obtained from investigations in the basal ganglia and limbic regions [52, 53] due to the high abundance of A₁Rs, A_{2A}Rs, D₁Rs, and D₂Rs in these areas and their involvement in the pathology of Parkinson’s disease. The antagonistic interaction of combined receptor activation seems to distinguish between adenosine and dopamine receptor subtypes. While A₁Rs communicate mainly with the D₁R subtype in strionigral-striopenduncular neurons, A_{2A}R and D₂R interaction occurs in striopallidal neurons. Studies on mice and monkeys pretreated with MPTP suggest that some degree of dopaminergic activity is needed to obtain adenosine antagonistic-induced motor activity. Furthermore, blockade of dopaminergic neurotransmission counteracts the antagonistic effect induced by adenosine [54]. Sufficient endogenous adenosine is present interstitially in the substantia nigra pars reticulata to control dopaminergic effects. The effects of adenosine are absent when dopaminergic influence is suppressed [53]. Thus, it seems that monotherapy with A_{2A}R antagonists may only be useful in the early stages of Parkinson’s disease, but could support a therapeutic treatment with dopamine agonists in advanced stages. A promising approach using these therapeutic strategies can be seen in istradefylline, an A_{2A}R antagonist that has since successfully passed clinical trials [55]. However, A₁R blockade may also contribute to an increased dopamine release but this effect seems without clinical relevance.

Relationship between A₁Rs and NPY

Neuropeptide Y (NPY) is one of the most abundant neuropeptides and exerts various functions on at least six GPCR subtypes (Y₁Rs–Y₅Rs, y₆Rs). Immunohistochemical investigations revealed the appearance of the Y₁R and Y₅R subtypes in the rat frontal cortex [56–58]. Activation of NPY receptors results in an inhibition of excitatory synaptic transmission, while a presynaptic influence on cortical neurons has been postulated [59]. NPY receptors affect pertussis toxin-sensitive G proteins, which inhibit adenylyl cyclase and decrease cAMP levels. Inhibitory and facilitating effects on K⁺ and Ca²⁺ mobilization have also been observed [60]. Receptor-receptor interactions between Y₁Rs have already been described, such as the antagonistic interaction with galanin receptors in the hypothalamus of the rat and their functional relevance for food intake. In contrast, a facilitatory interaction between the two receptors exists in the amygdala which may be of relevance for fear-related behavior [61].

In the CNS, A₁Rs and NPY receptors share some similarities in distribution. Both A₁Rs and Y₁Rs are located

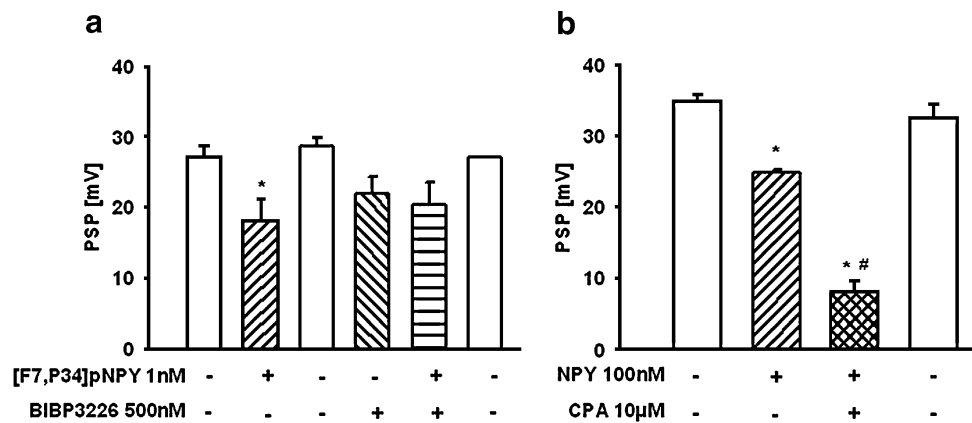


Fig. 1 Effect of the selective Y_1 agonist [F7,P34]pNPY as well as neuropeptide Y (NPY) alone and in combination with selective A_1 R agonist N^6 -cyclopentyladenosine (CPA) on the amplitude of postsynaptic potentials (PSPs) evoked by electrical field stimulation (0.2 Hz, 2 ms) in layer I of the rat cingulate cortex (Sichardt et al., unpublished results). Intracellular recordings were performed in rat brain slices using glass microelectrodes placed in pyramidal cells of layer V. **a** [F7, P34]pNPY superfused for 5 min inhibits reversibly the PSPs by $34.6 \pm$

8%. Y_1 antagonist BIBP3226 itself reduces the PSP by $23 \pm 10\%$, whereas [F7,P34]pNPY has no inhibitory effect in the presence of the antagonist. **b** NPY depresses PSPs by $28.6 \pm 0.7\%$. The combined superfusion of NPY and CPA resulted in an additional depression of the PSPs by $48.1 \pm 5\%$. The depressant effects of the two agonists were reversible during washout. Data are expressed as mean \pm SEM from $n=3$ independent experiments. * $p < 0.05$ significant vs control; # $p < 0.05$ significant vs NPY alone

on neurons of the prefrontal cortex, and their activation inhibits glutamatergic neurotransmission [62]. This is evidence for potential interaction between A_1 Rs and Y_1 Rs that may modulate long-term desensitization of A_1 Rs during pathophysiological situations. To investigate possible functional interactions, postsynaptic potentials (PSPs) were generated by electrical field stimulation on pyramidal neurons of layer V in the rat cingulate cortex as described by Brand et al. [35] and Hentschel et al. [36]. The Y_1 R agonist [F7,P34]pNPY inhibited the amplitude of PSPs. The inhibitory effect was reversible and reproducible, indicating that no desensitization appeared (Fig. 1a). An additional decrease in PSP amplitude was observed when NPY was superfused in combination with the A_1 R agonist CPA (Fig. 1b).

The additional inhibition induced by CPA was in the same range as that found with CPA alone ($48.1 \pm 5\%$ vs $55 \pm 3\%$). NPY inhibited PSPs after blocking CPA-mediated inhibitory effects by DPCPX. No significant changes existed before and after blocking A_1 Rs (Fig. 2). The results suggest that no interaction between A_1 Rs and Y_1 Rs exist. Each neuromodulator contributes to inhibitory regulation of excitatory neurotransmission. Regarding the desensitization of A_1 Rs but not of Y_1 Rs, this may be important under pathophysiological conditions with increased adenosine concentration in the synaptic cleft.

Interaction of A_1 Rs and P_2Y_1 Rs

P_2Y_1 Rs have been cloned and characterized in several species including human and rat, whereas mRNA was detected in various regions of the brain. The receptor subtype can be activated by ATP, but ADP as a degradation

product of ATP is a more potent endogenous agonist. Cellular signaling differs between A_1 Rs and P_2Y_1 Rs since A_1 Rs couple to $G_{i/o}$ and P_2Y_1 Rs to G_q/G_{11} . In fact, P_2Y_1 Rs can be assumed to exert stimulatory effects in cells. P_2Y_1 R signaling occurs in non-neuronal and non-muscular cell types, as well as on neurons in the CNS [63] where a colocalization of A_1 Rs and P_2Y_1 Rs was demonstrated immunohistochemically in rat brain cortex, hippocampus,

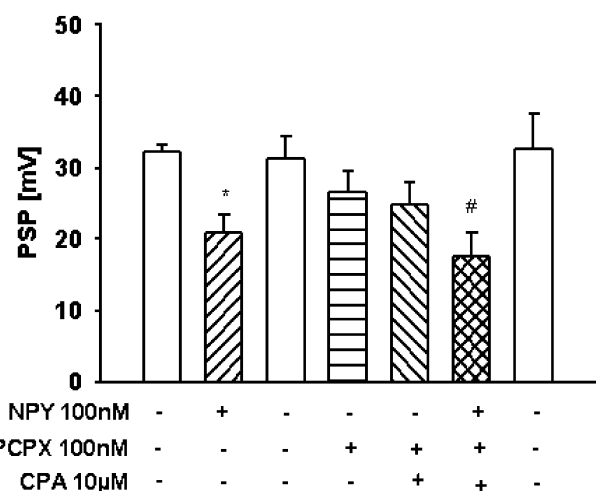


Fig. 2 Effect of neuropeptide Y (NPY) on the PSPs alone and in combination with the selective A_1 R agonist N^6 -cyclopentyladenosine (CPA) after preincubation with the selective A_1 R antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (Sichardt et al., unpublished results). The experimental procedure was similar to that shown in Fig. 1. In the presence of NPY, PSPs were decreased by $35.4 \pm 7\%$. Combined superfusion of NPY and CPA after preincubation with DPCPX decreased PSPs by $32.7 \pm 8\%$. The depression was reversible during washout. Data are expressed as mean \pm SEM from $n=5$ independent experiments. * $p < 0.05$ significant vs control; # $p < 0.05$ significant vs superfusion of CPA and DPCPX

and cerebellum [64]. In 1996, Ikeuchi et al. reported the activation of an undefined P2YR by adenosine in patch clamp and calcium imaging experiments on hippocampal neurons [65]. Furthermore, extensive heteromerization experiments have been conducted on cotransfected HEK293 cells using immunoprecipitation, Western blotting, and bioluminescence resonance energy transfer (BRET). Receptor binding experiments in combination with cAMP assays have also been described [64, 66–70]. These respective studies confirmed heteromerization associated with changes in the agonist binding and signaling compared to monomer properties. The binding for selective A₁R agonists was decreased while the A₁R antagonist binding remained unaffected. Interestingly, ADP binding was blocked by DPCPX but not by the P2Y₁R antagonist, suggesting an altered binding pocket on the A₁R/P2Y₁R complex. The G protein-coupling was sensitive to pertussis toxin and revealed a G_{i/o} status for the heteromer. Although colocalization of A₁Rs and P2Y₁Rs in several brain areas has been demonstrated, there is still a lack of functional investigations. Nevertheless, the physiological relevance of this interaction has been postulated as follows: costorage and release of ATP with neurotransmitters, such as glutamate or noradrenaline, occurs in the CNS [71–73]. ADP, the degradation product of ATP, acts as an A₁R agonist due to the activation of the A₁R/P2Y₁R complex and contributes to the inhibitory modulation of excitatory synaptic transmission via adenosine acting on A₁Rs. This interaction can be assumed as an additional mechanism for influencing and fine-tuning synaptic neurotransmission.

Functional interaction with ionotropic receptors

Neuronal excitability is regulated by voltage and ligand-gated ion channels. Ionotropic receptors also referred to as ligand-gated ion channels (LGICs) are a group of intrinsic transmembrane ion channels that open and close in response to binding of a chemical messenger, as opposed to voltage-gated ion channels or stretch-activated ion channels. Ion channels are regulated by a ligand and are usually very selective to one or more ions such as Na⁺, K⁺, Ca²⁺, or Cl⁻. These receptors located at synapses convert the chemical signal of presynaptically released neurotransmitter directly and very quickly into a postsynaptic electrical signal. Many LGICs are additionally modulated by allosteric ligands by channel blockers, ions, or membrane potential.

Nicotinic acetylcholine receptor serves as the prototypical LGIC [74] and consists of a pentamer of protein subunits with two binding sites, which, when bound, alter the receptor configuration and cause an internal pore to open. This pore, permeable to Na⁺ ions, allows them to flow down their electrochemical gradient into the cell. With

a sufficient number of channels opening at once, the intracellular Na⁺ concentration rises to the point at which the positive charge within the cell is sufficient to depolarize the membrane, and an action potential is initiated [75]. Many important ion channels are ligand-gated, and they show a great degree of homology at the genetic level. The LGICs are classified into three superfamilies; the first—the Cys-loop receptor family—is subdivided into the anionic GABA_A and glycine receptors on the one hand, and cationic 5-HT₃ serotonin and nicotinic acetylcholine receptors on the other. The second group—ionotropic glutamate receptors—consists of NMDA, kainate, and AMPA receptors. The third group covers the ATP-gated channels—the P2X receptors [76].

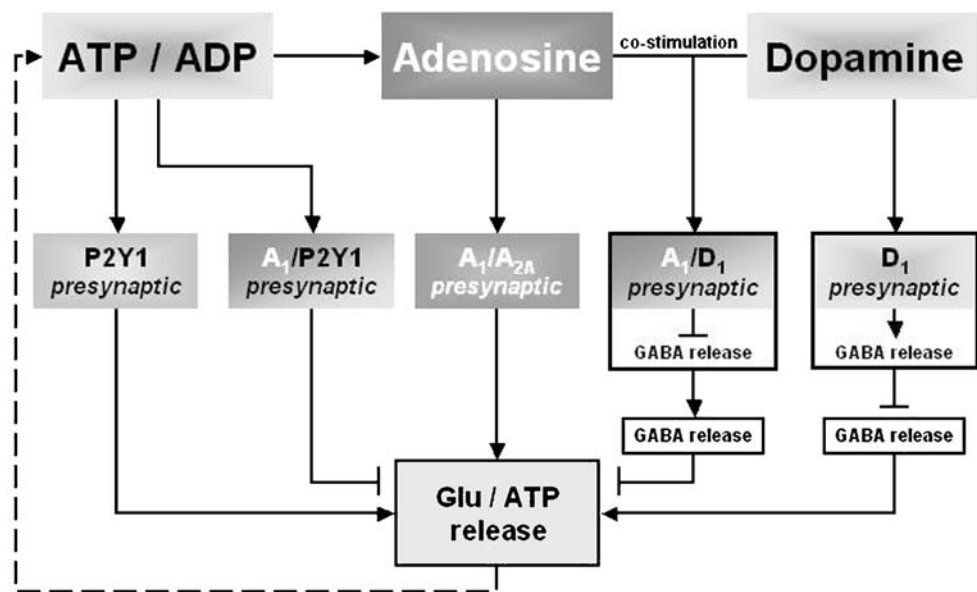
Adenosine is known to inhibit glutamatergic neurotransmission by activation of presynaptic A₁Rs [35]. This is probably due to reduction of the calcium influx, possibly by modulating both P/Q- and N-type presynaptic voltage-dependent calcium channels, which in turn controls transmitter release [77]. Furthermore, A₁Rs have long been known to mediate neuroprotection by reduction of excitatory effects at the postsynaptic level [10, 78, 79]. In addition to its direct presynaptic and postsynaptic actions on neurons, A₁R interaction with NMDA [80–82, 84], GABA_A [85–88], and P2X receptors [80, 89–91] contributes to fine-tuning neuromodulation via adenosine.

Interaction between A₁Rs and NMDA receptors

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. In most brain areas, glutamate mediates fast synaptic transmission by activating ionotropic receptors of the AMPA, kainate, and NMDA type. Additionally, NMDA receptors play a critical role in synaptic plasticity, synaptic development, and neurotoxicity. Recent studies suggest that some NMDA-mediated actions are altered or mediated by adenosine. Synaptic currents mediated by glutamate in rat substantia nigra pars reticulata neurons were reduced by adenosine acting via A₁Rs. The inhibitory action was not mediated by a postsynaptic site since adenosine did not block currents evoked by local application of glutamate [86].

NMDA is known to increase the extracellular level of adenosine via bidirectional adenosine transporters or from released adenine nucleotides degraded by a chain of ectonucleotidases [92, 93]. On the other hand, endogenous adenosine present in the extracellular fluid of hippocampal slices tonically inhibits NMDA receptor-mediated dendritic spikes as well as AMPA/kainate receptor-mediated synchronized EPSPs by activation of A₁Rs in CA1 pyramidal cells [81]. In line with these results, it has been shown that the tonic activation of A₁Rs by ambient adenosine depressed field potentials in the striatum. The effect of adenosine in

Fig. 3 Schematic representation of possible interactions of A_1 Rs with metabotropic receptors. Heteromerization between presynaptically located A_1 Rs and A_{2A} Rs, D_1 Rs, and $P2Y_1$ Rs causes changes in influencing glutamate release by adenosine. Cross talk between A_1 Rs and $P2Y_1$ Rs contributes mainly to triggering fast attenuation of transmitter release, whereas ADP acts as a ligand on the heteromer. During elevated adenosine levels, A_{2A} R signaling becomes dominant in the A_1 R/ A_{2A} R complex, providing enhancement of glutamate release. The A_1 R/ D_1 R heteromer requires both adenosine and dopamine to be activated and inhibits transmitter release

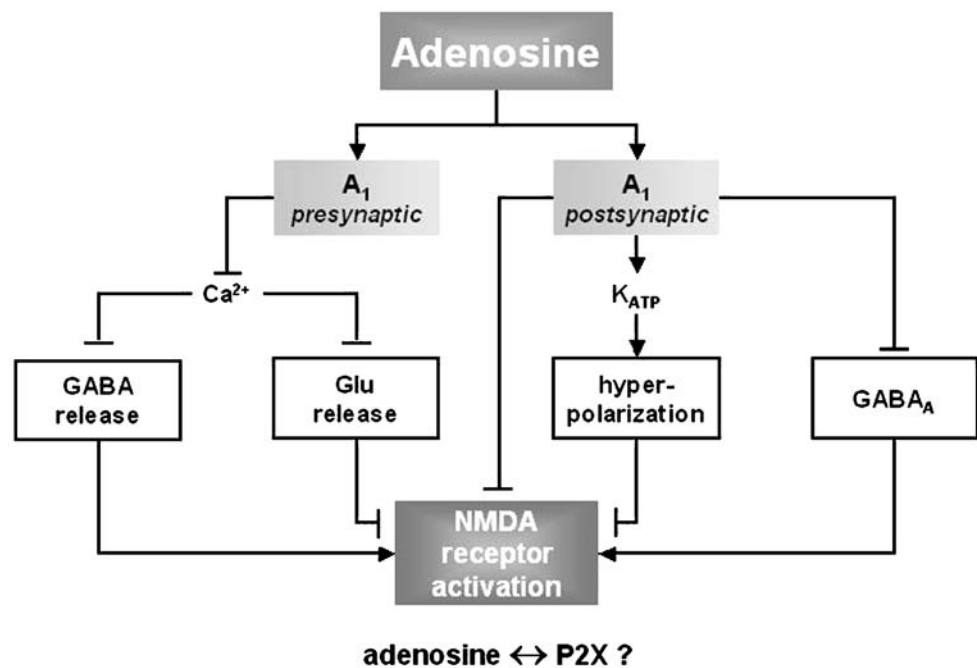


the striatum [84] or hippocampus [94] has not been found in A_1 R knockout mice and clearly demonstrates the involvement of A_1 Rs. The involvement of A_1 Rs was also supported by experiments using the selective receptor ligand 2-CA. In isolated rat hippocampal pyramidal cells [95] and in bipolar cells of the retina [96], 2-CA decreased inward currents induced by iontophoretic application of NMDA.

Another interesting interaction concerns NMDA preconditioning to protect against glutamate neurotoxicity. The A_1 R antagonist 8-CPT has been shown to prevent neuroprotection evoked by NMDA preconditioning against

glutamate-induced cellular damage in cerebellar granule cells [83]. In this study, the functionality of A_1 Rs was not affected by NMDA preconditioning, but this treatment promoted A_{2A} R desensitization in concert with A_1 R activation [83]. These results are in line with other studies indicating that adenosine downregulates excitatory and inhibitory synaptic transmission in several brain areas through activation of A_1 Rs and A_{2A} R [97, 98]. Furthermore, activation of A_1 Rs mediates reversal of long-term potentiation (LTP) produced by brief application of NMDA in hippocampal CA1 neurons [99].

Fig. 4 Schematic representation of possible interactions of A_1 Rs with ionotropic receptors contributing to the fine-tuning of neurotransmission. Adenosine acting via presynaptic A_1 Rs may attenuate the influx of Ca^{2+} through voltage-dependent calcium channels and thus decrease the release of glutamate and GABA, which inhibits or facilitates the activation of postsynaptically located NMDA receptors, respectively. Adenosine acting through postsynaptic A_1 Rs may activate K_{ATP} , which leads to hyperpolarization of postsynaptic neurons and inhibits directly the activity of NMDA and $GABA_A$ receptors



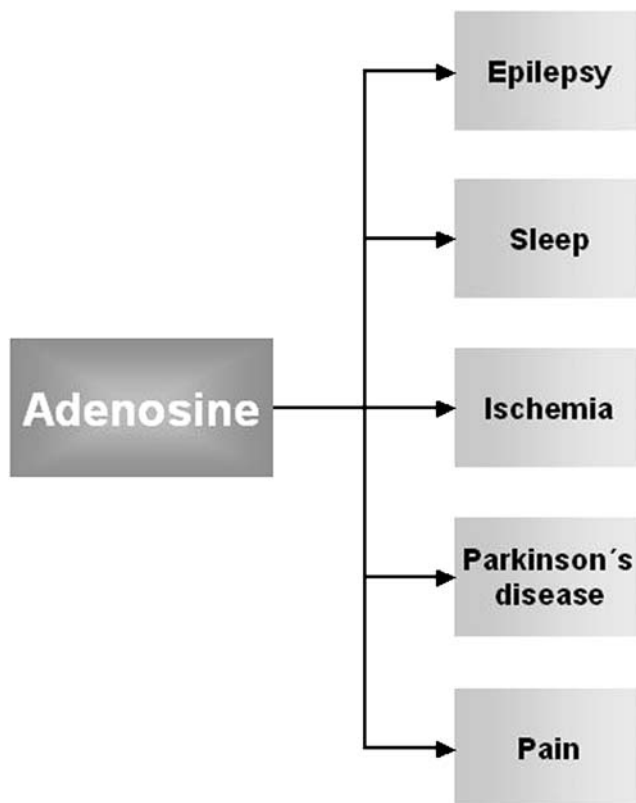


Fig. 5 Neurological disorders where A_1R interactions may play a role

Taken together, there are several ways in which adenosine may interact with NMDA-induced cellular events. Adenosine can affect glutamatergic transmission via both presynaptic and postsynaptic mechanisms by activating A_1R s [78]. NMDA receptors and A_1R s interact to down-regulate glutamate release presynaptically in pyramidal cells of the cingulate cortex [35], neurons of the hippocampus [100], and striatal neurons [84]. Another putative mechanism is related to postsynaptic A_1R s; adenosine elevates the threshold to open NMDA receptor-operated channels by antagonizing membrane depolarization [101].

Interaction between A_1R s and $GABA_A$ receptors

Fast synaptic inhibition in the brain and spinal cord is largely mediated by $GABA_A$ receptors that are also targeted by drugs such as benzodiazepines, barbiturates, neurosteroids, and some anesthetics. The modulation of their function will have important consequences for neuronal excitation [102]. One accepted means of modifying the efficacy is a functional interaction with adenosine. Adenosine may have an effect on either presynaptic GABA release in interneurons and/or on postsynaptic $GABA_A$ receptors in projection neurons. The site of action may be studied electrophysiologically by inducing fast inhibitory postsynaptic potentials (IPSPs) or application of GABA directly onto the cell.

Adenosine and selective A_1R agonist CHA reduced the amplitude of the fast IPSP in lateral amygdala slice preparations. The effect of CHA was blocked by DPCPX, indicating the involvement of A_1R s. Additionally, adenosine did not block currents evoked by local application of GABA [85]. Thus, the modulatory effect of adenosine on the GABAergic neurotransmission appears to take place on a presynaptic site by inhibiting GABA release from nerve terminals [85, 86]. The assumption that the activation of A_1R s can presynaptically modulate inhibitory postsynaptic responses agrees with findings in several brain areas, such as the thalamus [87], suprachiasmatic and arcuate nucleus [88], and substantia nigra pars compacta [86].

There is some evidence that activation of A_1R s is also involved in $GABA_A$ receptor downregulation, implying a facilitation of the neurotransmission on a postsynaptic site. GABA but not adenosine evoked an inward current in rat sacral dorsal commissural neurons (SDCN). The GABA-induced current was significantly reduced by adenosine, CHA and DPCPX, but not selective ligands for A_2AR s, mimicked or blocked the inhibitory effect of adenosine, respectively [103]. Adenosine and muscimol induced a concentration-dependent reduction in the amplitude of population potentials in hippocampal slices. Additionally, adenosine potentiated the ability of muscimol to inhibit evoked potentials, which were blocked by the A_1R -selective antagonist 8-CPT. The effects of adenosine as well as muscimol were reduced by the chloride channel blocker DIDS, indicating the ability of adenosine to regulate the $GABA_A$ chloride channel by activation of A_1R s [104].

Sebastiao's group studied the mechanisms by which GABA modulates adenosine-mediated effects and found that endogenous GABA exerts an inhibitory effect through $GABA_A$ receptors via a predominant adenosine-mediated action in the hippocampus. The authors concluded that there is an A_1R -mediated ability to inhibit synaptic transmission [19]. Further, this study showed that the blockade of GABAergic inhibition induced the release of NO, which was able to potentiate the inhibitory action of adenosine. They therefore suggested that the modulation of the A_1R -mediated response by activation of $GABA_A$ receptors occurs indirectly via NO [19].

Activation of $GABA_A$ receptors is effective in limiting neuronal ischemic damage [105] and endogenous adenosine that arises during hypoxia, and acts neuroprotectively partly by activating A_1R s [36]. Therefore, the contribution and potential interactions of GABA and adenosine as modulators of synaptic transmission during hypoxia has been investigated. Activation of A_1R s inhibits the release of GABA from the ischemic cerebral cortex in vivo [106]. In contrast, the administration of an A_1R agonist in the hippocampus failed to affect the release of GABA during ischemia [107]. In the light of these controversial results,

the role of the two neuromodulators during hypoxia was investigated in the CA1 area of rat hippocampal slices using selective A₁R antagonists [108]. Indeed, activation of A₁R and GABA_A receptors is partly involved in the inhibition of synaptic transmission during hypoxia. The action of GABA becomes evident when A₁Rs are blocked. Regarding the desensitization of A₁Rs during hypoxia [109, 110], it may be assumed that GABA_A-mediated inhibition of the synaptic transmission is evident when the A₁R is desensitized or downregulated [108].

Comodulation by A₁Rs and GABA_A receptors was also suggested in acute cerebellar ethanol-induced ataxia. Using GABA_A and A₁R agonists and antagonists, respectively, a functional similarity between GABA_A receptors and A₁Rs has been shown even though both receptor types are known to couple to different signaling systems [111]. This provides conclusive evidence that A₁Rs and GABA_A receptors both play a comodulatory role in ethanol-induced cerebellar ataxia without any direct interaction.

Functional interaction between A₁Rs and P2X receptors

P2X receptors are ligand-gated ion channel receptors; seven subunits (P2X₁-P2X₇) have been identified [63]. The P2X receptor subunits show many differences in localization, pharmacology, kinetics, and signaling pathways [112, 113]. The P2X₁ to P2X₆ receptors have 379–472 amino acids, with a predicted tertiary structure of transmembrane segments, a large extracellular loop and intracellular C and N termini. The P2X₂, P2X₄, and P2X₄/P2X₆ receptors appear to be the predominant neuronal types [91]. These subunits may occur as homooligomers or as heterooligomeric assemblies of more than one subunit. The P2X₇ receptor has a similar structure, but with a much larger intracellular C terminus. This contrasts strikingly with any of the other known ligand-gated ionotropic receptors [114]. P2X₇ subunits do not form heterooligomeric assemblies, but are involved in mediating apoptosis and necrosis in glial cells and possibly neurons.

Interaction between adenosine receptor-mediated and P2 receptor-mediated effects have been shown to occur in neuronal and non-neuronal cells [80]. Both adenosine and ATP induce astroglial cell proliferation and formation of reactive astrocytes [89]. In hippocampus, adenosine and ATP are released on stimulation and are potent neuronal transmission inhibitors [115, 116]. It should be pointed out that the interpretation of effects induced by both is difficult since ATP is degraded enzymatically to adenosine [38]. Adenosine is formed by extracellular catabolism of released ATP via the ectonucleotidase pathway [90, 117]. The role of the ectonucleotidases in forming adenosine is difficult to study since this system is extremely efficient, and it is difficult to block an enzyme system. The experimental

paradigm used by Cunha et al. [118] demonstrates that ATP has to be converted outside the cell into adenosine to exert its inhibitory effects on hippocampal synaptic transmission. The inhibitory effect of ATP was not modified by the P2 receptor antagonist suramin, but was attenuated by the ecto-5'-nucleotidase inhibitor and was nearly prevented by the adenosine A₁R antagonist DPCPX, whereas dipyrindamole, an inhibitor of adenosine uptake, potentiated the inhibitory effect of ATP [118]. These results offer evidence for localized catabolism of adenine nucleotides followed by substrate channeling to A₁Rs. This localized catabolism may mask the adenosine-mediated ATP effect [119]. Recently it was demonstrated that the exogenous application of ATP or ATP χ S reduced the hippocampal neurotransmission. The inhibitory effect was blocked by the selective A₁R antagonist DPCPX and was potentiated by different ecto-ATPase inhibitors [120]. These results suggest that the synaptic inhibition may consist of an inhibitory purinergic component of ATP itself in addition to degradation to adenosine.

Interaction with neuronal ATP-sensitive K⁺ channels

ATP-sensitive K⁺ channels (K_{ATP}) are widely expressed in the cytoplasmic membrane of neurons and couple cell metabolism to excitability [121]. These channels are regulated by the intracellular ATP/ADP ratio [122] and modulated by many endogenous mediators, including adenosine, via A₁Rs. Activation of A₁Rs inhibited the activity of inspiratory neurons in the brainstem by opening K_{ATP} in neonatal mice [123]. A₁R stimulation promotes K_{ATP} activity in principal dopamine neurons in the substantia nigra pars compacta [124] and hippocampus [125]. In contrast, one recent study has demonstrated that adenosine induces internalization of K_{ATP} resulting in a decrease in K_{ATP}-mediated response in the hippocampus [126]. The discrepancy might be due to the additional activation of A_{2A}Rs by adenosine located in hippocampus, but not in the substantia nigra pars compacta [127]. In addition to the inhibitory effect on the presynaptic site, the activation of A₁Rs acts as an inhibitory modulator to electrical activity on the postsynaptic site, and this effect has been attributed to enhancement of K_{ATP} activity. The modulating effect on the membrane potential may differ depending on the brain regions, as neuronal K_{ATP} is heterogeneous in different neurons.

A₁R interactions—new approaches for neurological disorders

By activation of its receptors, adenosine regulates many pathophysiological processes, particularly in excitable

tissues of the brain (Fig. 3). Its widespread functions in the body include regulation of seizure susceptibility [128, 129], neuroprotection [40], regulation of pain perception [130], sleep induction [131], and involvement in Parkinson's disease [132]. There is increasing evidence that the functional interaction of A₁Rs with other neuronal receptors may contribute to fine tuning in synaptic transmission, and A₁R agonists may represent a useful therapeutic approach for the treatment of some neurological disorders by regulating homeostasis in transmitter systems. However, the use of A₁R agonists has not proved clinically useful due to mainly cardiovascular side effects as well as low brain permeability. Pioneering experimental approaches have been evaluated using focal drug delivery in epilepsy models. One experimental study has used intraventricular implantation of an adenosine-releasing synthetic polymer [128]. In a later study, Guttlinger et al. [133] used encapsulated C2C12 myoblasts that were engineered to release adenosine by disruption of their adenosine kinase gene [133]. The local delivery of adenosine by implanted cells appears to be a promising strategy for the control not only for affecting seizure activity but also other neurodegenerative diseases with dysregulated synaptic neurotransmission.

Concluding remarks

As a consequence of its ubiquitous distribution and because of its linkage to the energy pool, adenosine has evolved as an important messenger in extracellular signaling. Modifications in extracellular adenosine levels with subsequent alterations in the activation of its receptors interferes with the action of other receptor systems. Figure 4 summarizes possible interactions of A₁Rs with metabotropic receptors, Fig. 5 shows the interactions between A₁Rs and ionotropic receptors as well as with K_{ATP}, and Fig. 3 shows the neurological disorders where A₁R interactions may play a role.

There is evidence that various regulatory mechanisms exist as well as multiple mechanisms that act independently of each other on the same cell depending on the brain region and cell type. The overaction and redundancy principle ensures transmitter homeostasis under pathophysiological conditions in a special time window. The function of adenosine receptors in the regulation of the synaptic transmission is complex.

The key receptor in regulation of the neuronal transmission may be the A_{2A}R, whereas the interaction of the A₁R with metabotropic and ionotropic receptors as well as with K_{ATP} serves as fine-tuning to inhibit synaptic transmission, as mentioned by Sebastiao and Ribeiro [80]. A₁Rs may play a nonessential role in normal physiology as demonstrated in mice lacking the A₁Rs [134]. However, they play an important protective role under pathophysiological

conditions especially during hypoxia. The activation initiates a fast inhibition of the glutamatergic neurotransmission and the receptor interactions may contribute to its maintenance or can support the A₁R-mediated effects.

Most of our knowledge on receptor-receptor interactions involving the A₁R results from experiments on cell cultures, slice preparation or, to a lesser extent, from *in vivo* experiments where regulation can be studied in principle or new drug targets can be characterized. These findings may contribute to a better understanding of disturbances in transmitter homeostasis. As our understanding of the complexity of receptor signaling and interaction develops, we may well gain new perspectives in new drug development. The clinical relevance of the testing models has often been questioned, however. Discordance between studies on cells and animal and human studies may be due to bias or failure of models to mimic clinical disease to an adequate degree. There are new techniques such as neuroimaging, nanotechnology, siPCR, and new selective receptor ligands that will help to overcome some of these aspects in the near future.

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