Modulation of excitatory neurotransmission by neuronal/glial signalling molecules: interplay between purinergic and glutamatergic systems

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Abstract Glutamate is the main excitatory neurotransmitter of the central nervous system (CNS), released both from neurons and glial cells. Acting via ionotropic (NMDA, AMPA, kainate) and metabotropic glutamate receptors, it is critically involved in essential regulatory functions. Disturbances of glutamatergic neurotransmission can be detected in cognitive and neurodegenerative disorders. This paper summarizes the present knowledge on the modulation of glutamate-mediated responses in the CNS. Emphasis will be put on NMDA receptor channels, which are essential executive and integrative elements of the glutamatergic system. This receptor is crucial for proper functioning of neuronal circuits; its hypofunction or overactivation can result in neuronal disturbances and neurotoxicity. Somewhat surprisingly, NMDA receptors are not widely targeted by pharmacotherapy in clinics; their robust activation or inhibition seems to be desirable only in exceptional cases. However, their fine-tuning might provide a promising manipulation to optimize the activity of the glutamatergic system and to restore proper CNS function. This orchestration utilizes several neuromodulators. Besides the classical ones such as dopamine, novel candidates emerged in the last two decades. The purinergic system is a promising possibility to optimize the activity of the glutamatergic system. It exerts

not only direct and indirect influences on NMDA receptors but, by modulating glutamatergic transmission, also plays an important role in glia-neuron communication. These purinergic functions will be illustrated mostly by depicting the modulatory role of the purinergic system on glutamatergic transmission in the prefrontal cortex, a CNS area important for attention, memory and learning.

Keywords Neuromodulation · Nucleotides · Glutamate · Prefrontal cortex

Introduction

Glutamate is the primary neurotransmitter of excitatory synapses in the CNS [1]. It acts pre- and postsynaptically by activating glutamate receptors. These receptors are responsible for excitatory neurotransmission and are pivotal elements of complex systems underlying synaptic plasticity, learning, memory and other fundamental events/functions in neurophysiology [2].

Glutamate receptors are sorted into two major classes: ionotropic glutamate receptors (ligand-gated ion channels) and metabotropic (G protein-coupled) glutamate receptors [3, 4]. Glutamate-activated ion channels were named for selective agonists by which they are activated: *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate [4]. Metabotropic glutamate (mGlu) receptors are further subdivided into three groups of eight subtypes: group I consisting of mGlu1 and mGlu5 (coupled to G_q/G_{11}), group II consisting of mGlu2 and mGlu3, as well as group III consisting of mGlu4, mGlu6, mGlu7 and mGlu8 (members of both latter groups are coupled to G_i/G_o) [3].

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Rapid synaptic excitation in the CNS is mediated primarily by the activation of postsynaptic ionotropic glutamate (AMPA and NMDA) receptors. To a smaller extent, P2X purinergic and nicotinic acetylcholine receptors also contribute to the excitatory postsynaptic currents [4, 5]. AMPA and NMDA receptors play different, well-defined roles in excitation. AMPA receptors are considered to be the primary mediators of fast neurotransmission under resting conditions. NMDA receptors, based on their unique properties, function as coincidence detectors (detecting the coincidence of glutamate release and postsynaptic depolarization) for the induction of long-term synaptic changes [6, 7]. Specifically, NMDA receptor activation is controlled by strong voltage dependence due to receptor channel blockade by Mg^{2+} at the resting membrane potential [8, 9]. Modulation of this receptor type will be in the focus of our review, whereas AMPA and kainate receptors will be outlined only briefly. In the first part of this review, subsequent to their characterization, and a brief resume of their role in health and disease, fine-tuning of NMDA receptors by neuromodulators will be discussed. In the second part, the purinergic neuromodulation of the glutamatergic system will be summarized and exemplified by delineating multiple modulatory influences of purines on the glutamatergic excitatory transmission in the prefrontal cortex (PFC). Purinergic mechanisms involved in synaptic plasticity, neurophysiology and neuropsychopathology will be reviewed and perspectives of the therapeutic utilization of the growing knowledge on purinergic neuromodulation will be depicted.

Ionotropic glutamate receptors

AMPA and kainate receptors

AMPA receptors are Na^+ and K^+ (in certain cases Ca^{2+}) permeable ion channels being the major determinants of the rapid component of excitatory synaptic currents in the brain. They show fast activation and deactivation kinetics as well as rapid desensitization. Therefore, glutamate challenge of AMPA receptors triggers a brief, rapidly rising conductance that decays also very quickly (1–2 ms) [1, 4].

Four subunits (GluA1–GluA4) form a tetrameric AMPA receptor structure (typically dimers of dimers) with a central pore. Alternative splicing at the flip/flop exon and RNA editing result in subunit variants with distinct channel properties. For instance RNA editing of GluA2 subunits known to occur in the adult brain prevents Ca^{2+} flux through the channel [10]. The major domains of the AMPA receptor subunits are the following ones: the extracellular N-terminus, the extracellular ligand-binding domain, the transmembrane domains and the intracellular C-terminus. The receptor subunits interact with transmembrane AMPA regulatory proteins (TARPs), influencing the synthesis, trafficking and localization of

AMPA receptors at the cell surface as well as their functional properties (channel conductance, open probability, activation, deactivation and desensitization characteristics). Further accessory proteins such as cornichon proteins regulate the AMPA receptor functions for instance by determining their subunit composition [4, 11–13].

AMPA receptors are expressed throughout the brain and are enriched in the postsynaptic membrane of the excitatory synapses. The surface localization of AMPA receptors shows a very dynamic pattern such as intense trafficking and recycling as well as lateral mobility between synaptic and extrasynaptic regions. Changes in AMPA receptor number is of great significance for CNS functions, for instance in shaping synaptic plasticity [14].

AMPA receptors are the subjects of not only transcriptional and translational control but also post-translational regulation. Several phosphorylation sites have been recognized on the C-terminal domains of the receptor subunits, and phosphorylation at these sites may influence receptor characteristics from membrane insertion, to open probability and other channel functions [4]. Phosphorylation of GluA1 subunit by calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase A (PKA) may result in changes in synaptic trafficking of AMPA receptors and may contribute to long-lasting changes of synaptic transmission [4, 15, 16].

Kainate receptors are also widely expressed in the brain and are important mediators of the pre- and postsynaptic actions of glutamate. However, much less is known about their physiological functions in comparison with AMPA and NMDA receptors. In some regions (e.g. thalamocortical synapses, somatosensory cortex), postsynaptic kainate receptors are involved in the generation of the synaptic current. Presynaptic kainate receptors can modulate the release of neurotransmitters (e.g. GABA or glutamate). In addition, kainate receptors may be involved in the integration and maturation of neural circuits and other developmental events [17].

Similar to other ionotropic glutamate receptors, kainate receptors are tetramers of subunits (GluK1–5). GluK1–GluK3 can form homomeric or heteromeric functional ion channels, while GluK4 and GluK5 subunits do not produce functional homomeric channels and participate in heteromeric receptors only as partners of GluK1–GluK3 subunits [18].

A special feature of kainate receptor channels is the possibility of noncanonical signalling, i.e. signalling by activation of a G protein, and thereby behaving like a metabotropic receptor [19, 20]. Thus, kainate receptors cannot be considered simply as ligand-gated ion channels. Rather, they seem to exert dual signalling (i.e. canonical, noncanonical) functions; this can be one of the reasons for their diverse actions. The metabotropic signalling was observed in many cell types and in different CNS regions, and it may be involved in the presynaptic control of neurotransmitter release or the postsynaptic regulation of neuronal excitability [17].

Several proteins have been identified which can interact with kainate receptors, regulating receptor trafficking and surface expression. For instance, neuropilin tolloid-like (Neto) auxiliary proteins exert an important influence on kainate receptor gating, desensitization and synaptic targeting [13, 21]. Phosphorylation of receptor subunits (for instance C-termini on GluK5 by CaMKII) also modulates channel properties [22].

Kainate receptor-mediated synaptic responses are smaller in amplitude than those mediated by AMPA receptors and show slow activation and deactivation kinetics [23]. This slow kinetics may provide integrative capacities to information transfer [17]. The kainate response may integrate synaptic inputs across a much longer period of time than those of the AMPA response, enabling temporally fewer correlated inputs to summate [24]. Temporal integration of excitatory circuits is modulated by various mechanisms. Postsynaptic kainate receptors can produce long-lasting depolarization or they can inhibit slow afterhyperpolarization currents via noncanonical signalling pathways [17]. Since activity-induced plasticity may result in a reduction of the kainate receptor-mediated component of the postsynaptic current, it may lead to a reduced ability of synapses to summate temporally [25].

NMDA receptors

NMDA receptors are involved in fundamental functions of neural circuits. For instance, activation of postsynaptic NMDA receptors enhances the depolarization provided by the activation of co-localized AMPA receptors. NMDA receptor function is designed to detect the coincidence between presynaptic activity (glutamate release) and postsynaptic activity (depolarization of the postsynaptic membrane) and initiate longer lasting changes of the synaptic activity [26]. Besides their postsynaptic localization, NMDA receptors can also be found in presynaptic localization, extrasynaptically, and in glial cells, being critically involved in CNS functions [27–29].

Like other ionotropic glutamate receptors, NMDA receptors are transmembrane proteins forming a central pore. Seven subunits (GluN1, GluN2A–GluN2D and GluN3A–GluN3B) have been hitherto identified [30, 31]. Alternative splicing (for instance in case of GluN1 eight splice variants) can further diversify the receptor subunit composition [32].

Most NMDA receptors are heterotetrameric complexes typically composed of two GluN1 and two GluN2 subunits [33]. Partnering of GluN1 or both GluN1 and GluN2 subunits with GluN3 subunits was also observed in recombinant systems. The subunit composition may have a significant impact on the functional properties of NMDA receptors. For instance, if the GluN3 subunit is incorporated into a functional channel in addition to GluN1 and GluN2 subunits, NMDA currents are reduced and the receptor is less sensitive to magnesium

blockade [34, 35]. The subunit composition may be specific for the localization of the NMDA receptor and may change during development. As an example, a basic concept is that GluN2A subunits are localized in synapses and GluN2B subunits occur in extrasynaptic regions of neurons [32, 36, 37]. Although this distribution of NMDA receptor subunits is supposed to have special importance, other data support the view that both GluN2A and GluN2B subunits are present at both sites [38, 39].

NMDA subunits contain a long extracellular N-terminal domain, a ligand binding domain (glutamate binds to GluN2 subunits and the co-agonist glycine binds to GluN1 and GluN3 subunits), a transmembrane domain (four membrane domains connected by short loops) and an intracellular C-terminal domain [32, 40]. The function of NMDA receptors is regulated by post-translational modifications and by protein binding partners. Serine/threonine phosphorylation sites of the C-terminal domains are substrates for protein kinases, CaMKII and other kinases [41]. Phosphorylation at these sites may increase NMDA currents and Ca^{2+} permeability of the channel [42–44]. Several G protein-coupled receptors (D1 dopaminergic, M1 muscarinic, metabotropic glutamate receptors—see below) have been reported to enhance NMDA receptor function via phosphorylation of the subunits. Involvement of the Src family of protein tyrosine kinases has been repeatedly observed in these facilitatory interactions [45, 46]. GluN2A and GluN2B subunits have palmitoylation sites in their C-terminal domains. Palmitoylation controls synaptic expression, internalization and trafficking of NMDA receptors. Ubiquitination is also proposed to be an important mechanism involved in dynamic regulation of NMDA receptor populations [27, 47].

Interaction of NMDA receptor subunits with cytoskeletal, scaffolding and signalling proteins influences their trafficking, clustering or surface expression [4, 48]. In the postsynaptic density, NMDA receptors form large macromolecular complexes partnering signalling molecules, kinases and phosphatases, adhesion proteins and other macromolecules [49]. Synaptic targeting and surface expression of NMDA receptors is controlled by interaction with PSD-95-like membrane-associated guanylate kinases (PSD-MAGUKs), such as PSD95 and SAP102 [27, 48, 50, 51].

Activation of NMDA receptors requires simultaneous binding of glutamate and glycine (or D-serine) [52]. The NMDA channel pore is blocked in a voltage-dependent manner by Mg^{2+} ; channel opening requires postsynaptic depolarization to relieve the Mg^{2+} blockade [8, 9]. Therefore, receptor activation is dependent on the coincident agonist (and co-agonist) binding and postsynaptic depolarization. These peculiar factors of NMDA receptor activation are critically involved in its physiological roles (e.g. synaptic plasticity) [26].

Activated NMDA receptors are permeable not only to Na^+ and K^+ but, unlike most AMPA receptors, show also high

Ca^{2+} permeability. Ca^{2+} influx through the channel initiates events critically involved in long-lasting changes of synaptic activity such as long-term potentiation (LTP) and long-term depression (LTD) [4, 53]. On the other hand, NMDA receptor overactivation can be harmful by stimulating Ca^{2+} -dependent cell death pathways resulting in excitotoxic neurodegeneration and apoptosis [54]. NMDA receptors containing GluN2A/GluN2B subunit composition show the highest Ca^{2+} permeability, while the presence of a GluN3 subunit in the receptor composition results in low Ca^{2+} permeability. Interestingly, GluN1–GluN3 stoichiometry causes other special characteristics; these NMDA receptors form purely glycine-activated excitatory channels [55].

The native NMDA receptor ligands are glutamate and glycine. Glutamate binds to GluN2; further endogenous ligands known to bind to the glutamate binding site are aspartate, homocysteate and cysteinesulfinic acid. A synthetic ligand, *N*-methyl-D-aspartate, also occupies the glutamate binding site at the GluN2 subunit and, as a selective agonist, plays an important role in the ionotropic glutamate receptor nomenclature [32]. The co-agonist glycine binds to the GluN1 subunit. In addition, serine and alanine are also agonists at the GluN1 subunit [4]. D-serine can be the primary co-agonist for synaptic, but not for extrasynaptic NMDA receptors in certain brain regions [56]. Interestingly, a synthetic serine analogue, D-cycloserine, acts as a GluN1 partial agonist [57].

NMDA receptor activation can be inhibited by competitive antagonists either at the glutamate binding site (e.g. 2-amino-5-phosphonopentanoate) or at the glycine binding site (e.g. 5, 7-dichlorokynurenic acid) [4]. The first subunit-selective NMDA receptor antagonist, ifenprodil, inhibits GluN2B-containing NMDA receptors as a negative allosteric modulator [58]. Phencyclidine, MK-801 (dizocilpine), ketamine, amantadine and memantine are use-dependent open channel blockers, sealing the receptor pore after channel opening. After channel closure, these compounds can be trapped in the pore, causing a long-lasting, slowly reversible blockade. Protons and divalent ions, such as extracellular Zn^{2+} , are also known to block the NMDA channel by multiple mechanisms, the latter in part in a voltage-dependent manner [4]. The significance of voltage-dependent blockade of NMDA receptors by extracellular Mg^{2+} was mentioned above. Endogenous polyamines, such as spermine and spermidine, have been reported to enhance NMDA receptor currents by both glycine-dependent and glycine-independent mechanisms [59]. For further details of allosteric modulation and pharmacology of NMDA receptors, please consult recent reviews [60–62].

Executive and integrative roles of NMDA receptor channels

The ionotropic glutamate receptors comprise the major excitatory system in the CNS. Furthermore, NMDA receptors are

crucially involved in the integrative properties of neurons, based on their unique features mentioned above, i.e. nonlinear amplification (Mg^{2+} blockade at resting potential) and high permeability to Ca^{2+} [6].

As it was pointed out above, NMDA receptors sense the coincidence between presynaptic activity (binding of the presynaptically released glutamate to the NMDA receptors) and postsynaptic activity (sufficient depolarization of the postsynaptic membrane to alleviate Mg^{2+} blockade) [26].

Long-lasting changes in synaptic strength such as LTP and LTD are considered to be the underlying mechanisms involved in memory storage, learning and thereby higher order cognitive functions [2, 53]. Postsynaptic AMPA and NMDA receptors are the major determinants of synaptic strength at excitatory synapses [1]. AMPA receptors mostly define the basal synaptic transmission, while NMDA receptors seem to be critically involved in the activity-dependent changes. As the voltage-dependent Mg^{2+} blockade is relieved due to the depolarization of the cell membrane (for instance by co-localized AMPA receptors), NMDA receptors will be triggered. As a consequence, intracellular Ca^{2+} raises, resulting in activation of kinases and phosphatases, which influence the phosphorylation state of various macromolecules, including the non-NMDA (AMPA, kainate) receptors. The ensuing changes in AMPA receptor conductance and/or density reset the AMPA-mediated responses. Along with remodelling of postsynaptic densities and signal transduction mechanisms as well as formation of new synapses, these changes are supposed to be key mechanisms underlying LTP, LTD, synaptic plasticity and memory formation [45, 63].

Recent data indicate that previously obscure mechanisms can further deepen the role of NMDA receptors in fine-tuning of information encoding and storage. In contrast to the original view of less dynamic NMDA receptors, recent evidence suggests that they not only modify the induction threshold of AMPA-mediated synaptic changes, but that NMDA receptors themselves are also subjects of regulation in an activity-dependent manner. The mechanism of this activity-dependent NMDA receptor plasticity might involve trafficking events as well as changes in channel properties such as Ca^{2+} permeability [6, 45].

Synaptic and extrasynaptic NMDA receptors

NMDA receptors are localized in the synaptic cleft, as well as at extrasynaptic sites. The balance between synaptic and extrasynaptic NMDA receptor activation is of great significance for proper neuronal functioning of the brain. The localization hypothesis of NMDA receptors conceives that synaptic NMDA receptors support cell survival, while extrasynaptic ones promote cell death [64]. Specifically, these two distinct NMDA receptor populations are supposed to trigger different intracellular downstream events. Synaptic NMDA receptor

activation enhances extracellular signal-regulated kinase 1/2 (ERK1/2) activity, cAMP response element-binding protein (CREB) phosphorylation and brain-derived neurotrophic factor (BDNF) expression, thereby promoting neuroprotection. On the contrary, extrasynaptic NMDA receptor activation resulted in opposite effects [64]. In contrast to synaptic NMDA receptors, extrasynaptic NMDA receptors can impede LTP formation [29, 65]. Selective activation of extrasynaptic NMDA receptors induces LTD instead [66].

The two receptor populations may contain receptors with different subunit composition (dominance of GluN2A containing receptors in the synapse versus dominance of GluN2B containing receptors at extrasynaptic sites—see above). The subunit composition of NMDA receptors seems to have a major influence on the direction of synaptic plasticity. The presence of GluN2A subunits promotes LTP, while GluN2B subunit is associated with LTD induction [67, 68]. Furthermore, subunit composition has been proposed to influence cell viability, GluN2A-type NMDA receptors being linked to cell survival, whereas GluN2B-type NMDA receptors to cell death, respectively [69, 70]. Conflicting with this view, recent data demonstrated that GluN2A subunit-containing synaptic NMDA receptors can be involved in excitotoxic cell death under certain conditions [29, 71].

The perisynaptic region may contain mobile NMDA receptors, which can diffuse between synaptic and extrasynaptic sites. Location on the plasma membrane may affect the function of NMDA receptors, and their dislocation may be involved in the pathology of neurological disorders [27, 72].

Glutamate and ‘gliotransmission’

Astrocytes have a variety of functions in the CNS such as (i) creation of the functional microarchitecture of the brain during development, (ii) creation of the glial-vascular interface (blood-brain barrier), (iii) maintenance of ionic homeostasis in the extracellular space, (iv) regulation of water homeostasis and extracellular space volume, (v) metabolic support of neurons and (vi) functional interaction with neurons by establishing neuronal glial networks [73]. The concept of ‘tripartite synapse’ suggests that astrocytes are in tight contact with neurons, and glial cells are endowed with the ability to control the activity and synaptic strength in neuronal circuits [74]. Neurons and glial cells communicate with each other: neuronal activity can result in the elevation of intracellular Ca^{2+} in glial cells via the stimulation of neurotransmitter receptors [75], and this Ca^{2+} elevation elicits the release of transmitters (‘gliotransmitters’) from astrocytes. Actually, astrocytes are neuroectodermal cells, which release identical transmitter molecules (including glutamate and ATP) by the same exocytotic machinery as neurons (see below). Therefore, the term ‘gliotransmitter’ might be misleading. Nevertheless, we continue to use this term (although in quotation marks) because of

its prevalence in the scientific literature and because mesodermal microglia has also been shown to release ATP exocytotically [76].

‘Gliotransmitters’ include amino acids (glutamate, D-serine), nucleotides (ATP, adenosine) and peptides (atrial natriuretic peptide, brain-derived neurotrophic factor) [77]. One of the proposed gliotransmitters is glutamate and the other one a co-agonist at NMDA receptors, D-serine; thereby, in a tripartite glutamatergic synapse, a complex glutamatergic communication takes place between presynaptic and postsynaptic neurons as well as glial cells. Glutamate is released from astrocytes, and glia is also involved in the re-uptake and inactivation of glutamate; thereby, glia participates in the control of extracellular (synaptic and extrasynaptic) glutamate levels and in the glutamate-mediated physiological and pathophysiological actions. Impaired glial cell activity may cause not only disturbances in synaptic plasticity, but it can also lead to increased glutamatergic activation and neurotoxicity, especially at extrasynaptic sites [78–80].

Newly, the concept of the tripartite synapse, consisting of the pre- and postsynaptic neuronal elements and the astroglial end-feet enwrapping the synapse, was extended by a further active player, the microglia (quad-partite synapse) [81]. Microglial cells, the resident immune cells of the CNS, are of mesodermal origin, in contrast to astroglia which derive from the neuroectoderm. Microglia have important roles to survey the extracellular environment during disease; however, more recently, their numerous functions in the uninjured brain have also been described. They actively engulf synapses and are involved in synaptic pruning during postnatal development [82]. In addition, they monitor neuronal activity and release themselves as ‘gliotransmitters’ such as ATP, which in turn releases glutamate from astrocytes and subsequently modulates neuronal activity through metabotropic glutamate receptors [83]. Moreover, stimulation of neuronal NMDA receptors triggers ATP release causing transient microglial process outgrowth surveying their environment [84].

It has been hypothesized based on tissue culture work that glutamate and ATP/adenosine release from astrocytes is by exocytotic machinery. Perhaps the strongest *in vivo* evidence in support of ‘gliotransmission’ was the development of a transgenic mouse line in which vesicular release could be specifically inhibited in astrocytes. In these mice, the formation of the SNARE complex between vesicles and the plasma membrane is inhibited by the expression of a dominant negative domain of the vesicle-associated membrane protein 2 (VAMP2) protein, which interferes with endogenous VAMP2 expression and thus prevents membrane fusion [85]. Cultured microglia has also been shown to release ATP by exocytosis [76]. However, cultured astrocytes and microglial cells are very plastic and therefore might not reflect the *in vivo* situation. Moreover, the expression of the dominant negative (dn)SNARE complex might be not totally restricted to

astrocytes, and even a small disruption of neuronal vesicular release has the potential to suppress glutamatergic transmission [86, 87].

In view of the purinergic P2-NMDA interactions, discussed in detail below, it is noteworthy that ATP has also emerged as a signalling molecule substantially involved in neuron-glia communication [88–90].

NMDA receptors in health and disease

NMDA receptors are inevitably needed for synaptic plasticity, learning and memory formation. However, the excessive activity of NMDA receptors can result in cell death. Hence, these receptors are implicated in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease, Huntington's chorea, ischemia/hypoxia, traumatic brain injury, epilepsy, neuropathic pain, Parkinson's disease, schizophrenia and mood disorders [4, 29, 30, 61, 78].

Therefore, NMDA receptors are considered to be promising targets to influence neuronal dysfunctions and to ameliorate the symptoms of various human diseases. Indeed, numerous substances have been designed to alter NMDA receptor activity as a therapeutic intervention. However, somewhat surprisingly, only few drugs targeting NMDA receptors are in the clinical practice [4, 91].

The problem is obvious: a direct enhancement or inhibition of NMDA receptors would be a logical approach to influence a pathological situation. However, a robust activation of NMDA receptors is unsafe, potentially leading to cell death and neurodegeneration. The proper therapeutic strategy could be the normalization of the NMDA function instead. Therefore, a better understanding of the complex functioning of NMDA receptors is of great significance. Especially, elucidation of mechanisms involved in fine-tuning of the receptor complex may unveil novel pharmacological targets. Selective modulators may have less profound, subtle effects on the system compared with direct inhibitors and may offer more suitable approaches to restore the physiological situation [26].

Fine-tuning of NMDA receptor channels

Adjustment of the activity of NMDA receptors can occur at several levels. Protons, multiple divalent ions and polyamines as modulators of the receptor activation have been mentioned above. Changes in the NMDA receptor activation level can be the consequence of the presynaptic action of neuromodulatory systems influencing the amount of the released glutamate. Considering that the receptor activation requires the binding of a co-agonist, modulation can also target the co-agonist substance level in the surrounding area by influencing glycine release or the activity of glycine transporters [92]. Modulatory influences can also target the co-agonist binding to the NMDA

receptor. An interesting observation is that glycine can be involved not only in the gating of this receptor, but it may have also a priming effect by permitting NMDA receptor internalization, when glycine concentration exceeds the basal physiological concentration [93].

Considering the voltage-dependent Mg^{2+} blockade of the NMDA receptors, it is not surprising that several modulators regulate NMDA receptor function via depolarization. Conversely, hyperpolarization of neurons for instance by activation of co-localized GABA receptors intensifies the Mg^{2+} blockade of NMDA receptors [26]. Regulation can also occur via intracellular signalling pathways. In addition, physical interactions between NMDA and other receptors may allow cross-talk via receptor linkages.

The modulatory influences often result in post-translational modifications such as altered phosphorylation, S-nitrosylation, palmitoylation and/or ubiquitination state of the receptor subunits. They may affect not only directly receptor signalling, but also by altering surface expression, internalization and trafficking of NMDA receptors; their interaction with cytoskeletal, scaffolding and signalling proteins; their translocation between synaptic and extrasynaptic sites, etc. [27, 47, 94, 95].

The following section summarizes the major influences of the neuromodulatory systems on the activity of NMDA receptors.

Modulation of NMDA receptors by metabotropic glutamate receptors

Postsynaptic metabotropic glutamate receptors (mGluRs) are frequently co-localized with NMDA receptors and are involved in their fine-tuning [96–99]. Group I (mGluR1 and mGluR5) receptors potentiate NMDA responses via various mechanisms including PKC-dependent and PKC-independent pathways [96, 97, 99–103]. Although most publications suggest the dominant role of group I mGlu receptors in the potentiation of NMDA currents, a possible modulatory role of group II (but not group III) mGlu receptors on NMDA currents is also possible in the rat prefrontal cortex [104, 105].

Besides numerous publications demonstrating potentiation of NMDA currents, inhibitory effects have also been sporadically reported in response to mGluR activation. In cultured mouse cortical neurons, membrane-delimited inhibitory modulation was suggested to take place [106]. In the hippocampus, NMDA (and AMPA) receptors were internalized in response to group I mGlu receptor activation [107].

Activation of mGlu receptors may result in a more complex regulation of NMDA receptor activity. For instance, in the rat visual cortex, potentiation in layers V and VI but depression in layer IV of NMDA responses were observed when mGlu receptors were activated [108]. In rat dorsal spinal horn, activation of group I mGlu receptors caused dual modulation. It

induced a long-lasting depression of primary afferent A-fibre-mediated monosynaptic excitatory postsynaptic potentials (EPSPs) and a long-lasting potentiation of polysynaptic EPSPs and of EPSPs in cells receiving C-afferent fibre input [109].

Modulation of NMDA receptors by dopamine

Dopamine is one of the major modulators of glutamatergic activity in the CNS influencing NMDA receptor function by multiple mechanisms involving pre- and postsynaptic actions [110].

Striatal glutamatergic nerve terminals are endowed with presynaptic D2 receptors inhibiting glutamate release [111, 112]. Other publications indicated that presynaptic D1 dopamine receptors may also be involved in the reduction of glutamate release, for instance in the nucleus accumbens [113, 114].

Postsynaptic dopamine receptors can both potentiate and inhibit NMDA receptor-mediated responses, depending on the dopamine receptor subtype involved in the interaction [110]. Low concentrations of dopamine (<50 μ M) via D1 receptor stimulation have been reported to facilitate, while high concentrations of dopamine (>50 μ M) via D2 receptor stimulation preferentially inhibit NMDA current responses in cortical neurons [115–117].

Dopamine receptor-mediated potentiation of NMDA currents has been repeatedly demonstrated in the cortex, striatum, nucleus accumbens and in other CNS regions [115, 116, 118, 119]. This enhancement is mediated via D1 receptor stimulation and subsequent activation of signal transduction systems regulating NMDA receptor activity. For instance, in the striatum, PKA, dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32), phosphorylation of NMDA receptor subunits and activation of voltage-gated Ca^{2+} channels have been reported to be involved in the potentiation of NMDA receptor function by dopamine [120, 121]. Involvement of PKA and increased intracellular Ca^{2+} were also demonstrated in the D1-NMDA receptor interaction in the prefrontal cortex [122]. Extracellular regulated kinase 1/2 (ERK1/2) is implicated in the potentiation of NMDA currents via D1 receptor activation in the prefrontal cortex and hippocampus [123]. In the hippocampus, activation of Fyn kinase and phosphorylation of GluN2B subunits were also observed as the underlying mechanisms of the D1-NMDA synergism [124]. Phospholipase C and PKC are implicated in the facilitation of NMDA signals by dopamine in the nucleus accumbens and in cortical pyramidal neurons [119, 125]. Jocoy et al. revealed that D1 receptor-induced enhancement of NMDA responses in the striatum was reduced in GluN1-KO mice. Furthermore, the absence of GluN2A subunits enhanced, while pharmacological blockade of GluN2B subunits decreased the D1-NMDA interaction [126].

Cepeda et al. reported first in 1993 that besides the potentiation of NMDA currents via D1 receptor activation, dopamine exerts additional actions in the striatum. Specifically, when selective D2 receptor agonists were tested, reduced NMDA responses could be observed [115]. This attenuation of NMDA responses by D2 receptor activation can be the consequence of reduced cAMP production and PKA activity. In the neostriatum, reduced neuronal activity by D2 receptor stimulation was dependent on the phospholipase C pathway, leading to a calcineurin-dependent reduction in L-type Ca^{2+} currents [110, 127]. In the prefrontal cortex, a more complex interaction has been suggested to take place: the inhibitory action of D2 receptors on NMDA-induced responses was mediated by GABAergic interneurons [122].

Postsynaptic interactions between dopamine and NMDA receptors can occur not only through signal transduction systems; direct physical receptor cross-talk may also occur. Protein-protein interactions between dopamine receptors and GluN1 or GluN2A subunits reduced NMDA receptor currents and attenuated NMDA-mediated excitotoxic signals, respectively [128]. Physical coupling between D2 receptors and C-terminal domains of GluN2B subunits has been found in striatal neurons reducing GluN2B phosphorylation and NMDA currents [129].

An additional level of the dopamine-NMDA interactions is that D1 receptor activation may affect the NMDA receptor subunit trafficking at the synaptic membrane. Namely, it promotes tyrosine phosphorylation-dependent surface insertion of the striatal NMDA receptor subunits into the membrane [130, 131]. Further, D1 receptors and NMDA receptors are co-localized in the postsynaptic density. It is proposed that dopamine and GluN1 subunits form oligomeric complexes yielding the possibility not only of the physical cross-talk, but also the reciprocal regulation of receptor trafficking, recruitment and internalization in the striatum [128, 132]. The interaction between D1 and GluN1 subunits was also reported in the hippocampus, where NMDA receptor subunits contribute to the synaptic stabilization of dopamine receptors. D1 receptor activation allows the lateral diffusion of NMDA receptors into the postsynaptic density, where they support the induction of LTP [133, 134].

Modulation of NMDA receptors by acetylcholine

Potentiation of NMDA currents by activation of muscarinic acetylcholine (mACh) receptors was repeatedly demonstrated, mostly in the hippocampus [26, 135–139]. Threefold mechanisms can be involved in this facilitatory effect: (1) depolarization of neurons by mACh receptors, for instance by inhibiting K^{+} channels, (2) activation of signal transduction systems with possible involvement of inositol trisphosphate and elevation of $[\text{Ca}^{2+}]_i$ [137, 139] and (3) activation of hippocampal calcineurin and alterations in the association of clathrin

adaptor molecule AP2 and the postsynaptic density protein PSD-95 with NMDA receptors and subsequent dynamin-dependent internalization of NMDA receptors [140]. In contrast, long-term amplification of NMDA transmission requiring combined CaMKII, PKC and Src kinase activity has been reported in the hippocampus, without detectable surface incorporation of NMDA receptors [136]. Cholinergic facilitation of NMDA-dependent synaptic changes was observed in other brain regions, such as in the rat visual cortex [141].

Presynaptic nicotinic acetylcholine (nACh) receptors are involved in the control of transmitter release including that of glutamate [142–144]. nACh receptors cooperate with coexisting presynaptic NMDA receptors in the facilitation of noradrenaline and dopamine release in hippocampal and striatal nerve terminals, respectively [145, 146]. In contrast, nicotine has been reported to evoke internalization of presynaptic NMDA receptors in dopaminergic terminals of the rat nucleus accumbens resulting in decreased NMDA-induced dopamine release [147]. On the other hand, nicotine pretreatment increased the responses of the NMDA receptors, which contain the GluN2A subunits, via activation of $\alpha 7$ -nACh receptor subtypes on the glutamatergic nerve endings of the nucleus accumbens [148].

Modulation of NMDA receptors by neurosteroids

Neurosteroids are known to be involved in the regulation of neuronal functions. An important part of these effects is the modulation of glutamatergic activity including fine-tuning of NMDA receptors. These modulatory effects consist of both facilitatory and inhibitory actions by various steroids; the precise mechanism of action has not been fully elucidated in many cases [91].

For instance, pregnanolone is a weak negative modulator of NMDA responses, suggested to be a use-dependent allosteric inhibitor [149, 150]. The inhibitory steroid binding site seems to be located on the extracellular part of the NMDA receptor, probably on the GluN2 subunit [150, 151].

In contrast, pregnenolone has been reported to be a powerful positive modulator of NMDA currents [152]. It has been suggested to increase the open probability of the NMDA channel by an allosteric action on the receptor, since it potentiated NMDA currents when it was pre-applied before the agonist [153]. Indirect, presynaptic actions, i.e. potentiation of glutamate release, may also contribute to the facilitatory actions of pregnenolone on excitatory neurotransmission, for instance by potentiating presynaptic nACh receptors [154, 155]. Further studies revealed that the actions of pregnenolone are even more complex on the NMDA receptor activity. Potentiation is not its sole effect; it may also inhibit NMDA currents (resembling the actions of pregnanolone). NMDA receptor subunit composition may be a critical issue in this context; potentiation occurs at GluN2A and B containing

receptors and inhibition at GluN2C and D [156]. Nevertheless, in most neurons (based also on their subunit composition), the overall effect of pregnenolone on NMDA receptors is usually potentiation.

Dehydroepiandrosterone also facilitated NMDA signalling and enhanced LTP in the hippocampus [157, 158]. Further, inhibition of the synthesis of the two latter neurosteroids reduced both NMDA receptor activity and LTP induction in the hippocampus, supporting the view that these steroids are continuously synthesized under physiological conditions and are required for normal synaptic transmission and plasticity [159].

Other modulators of NMDA receptors

Besides the neuromodulators briefly reviewed above, several further endogenous substances modulate NMDA receptor activity.

At the presynaptic site, glutamate release is regulated (facilitated or inhibited) by various ligand-gated ion channels, as reviewed by Khakh and Henderson [160]. Further, endogenous cannabinoids are known to act retrogradely on presynaptic CB1 receptors to suppress glutamate release [161]. Morphine via activation of μ opioid receptors as well neuropeptide Y through the activation of Y1 receptors inhibited the release of glutamate in the cerebral cortex [162, 163]. Nitric oxide facilitated glutamate release in cerebellar slices [164]. Neurotensin has been reported to enhance glutamate release via activation of NTS1 receptors, in rat cortex, striatum and substantia nigra [165]. $\alpha 1$ adrenoceptor activation resulted in presynaptic stimulation of glutamatergic transmission in the prefrontal cortex [166]. Similarly, the β adrenoceptor agonist isoproterenol facilitated glutamate release in the rat cerebral cortex [167]. The adrenergic system typically exerts multiple influences on the glutamatergic system including not only presynaptic but also postsynaptic modulatory effects. For instance, postsynaptic inhibition of NMDA currents by both $\alpha 1$ and $\alpha 2$ selective agonists has been reported [168, 169]. In contrast, $\alpha 1$ adrenoceptor activation enhanced NMDA-induced currents in rat prefrontal cortex [170].

As mentioned above, postsynaptic potentiation of NMDA responses (e.g. by ACh) can be the consequence of depolarization by relieving the Mg^{2+} blockade of the receptor. In contrast, GABA receptors causing hyperpolarization enhance Mg^{2+} blockade resulting in inhibition of NMDA receptors [26, 171].

Activation of G protein-coupled receptors may result in more precise, subtype-selective modulation of NMDA receptors. This is typically based on post-translational modification such as altered phosphorylation of the receptor. GluN1 subunits can be phosphorylated by PKA and PKC, regulating trafficking, clustering and calcium permeability of the NMDA receptors [172, 173]. Consensus phosphorylation sites on GluN2A subunits can also be phosphorylated; PKA alters

desensitization [174], while PKC and protein tyrosine kinases, such as Src kinase, cause potentiation of NMDA currents [124, 175, 176]. PKC and PKA are known to phosphorylate GluN2B subunits as well [177, 178]. CaMKII and casein kinase II are also identified regulators of the NMDA receptors by phosphorylating GluN2B subunits [179, 180].

Multiple actions of serotonin, one of the classical substances known to regulate the glutamatergic system, involve numerous modulatory mechanisms. Via activation of 5-HT_{1A} receptors, serotonin inhibits NMDA currents in the prefrontal cortex. This effect is targeted at the GluN2B subunit; various kinases such as CaMKII and MAPK/ERK are involved in this interaction involving microtubule/kinesin-based dendritic transport of NMDA receptors [181]. Activation of 5-HT_{2A/C} receptors, via ERK and β -arrestin-dependent pathway, opposed this action [182]. Further, various secreted soluble factors such as Wnt, brain-derived neurotrophic factor, transforming growth factor- β and fibroblast growth factors are also known to modulate NMDA receptor signals; most of these effects occur by influencing receptor phosphorylation (for a recent review, see [183]). Somatostatin potentiated NMDA receptor function via activation of inositol trisphosphate and PKC in the hippocampus [184]. NMDA receptor activity is triggered by insulin too; various kinases are involved in this insulin action [185]. GluN2A-containing receptors were potentiated in response to insulin solely by PKCs, while GluN2B-containing receptors were facilitated by both PKCs and tyrosine kinases [186]. The $\alpha 1$ adrenoceptor-mediated enhancement of NMDA currents mentioned above involves activation of phospholipase C and PKC in rat prefrontal cortex (PFC) [170].

Activation of EphB receptor tyrosine kinases by the cell surface-associated protein ephrin led to tyrosine phosphorylation and potentiation of NMDA receptors in primary cortical neurons [187]. Pituitary adenylate cyclase activating peptide (PACAP) stimulating PAC1 receptors, via PKC and Src kinase activation and phosphorylation of GluN2A subunits, potentiated NMDA currents in hippocampal neurons [124, 188]. In contrast, other authors suggested phosphorylation of GluN2B subunits and the involvement of the cAMP/PKA pathway in this interaction in dissociated hippocampal neurons [189]. Nevertheless, this modulatory action probably involves the release of the scaffolding protein RACK1 from NMDA receptors.

Somewhat surprisingly, plasmin and thrombin, the endogenous ligands of the protease-activated receptor-1 (PAR-1), caused robust potentiation of NMDA receptors in the hippocampus [190, 191]. Notably, the release of an astrocytic transmitter, namely glutamate, was involved in this interaction in rat hippocampal slices [191].

As mentioned above, other forms of post-translational modifications may also result in altered NMDA receptor activity. For instance, NMDA receptors can be modulated by S-

nitrosylation, and NO is an important inhibitory modulator of the NMDA receptor channels. Besides this direct modulatory action, NO influences the availability of the co-agonists and scaffolding proteins for the receptor [192].

Purines as novel modulators of the glutamatergic neurotransmission

One of the novel neuromodulatory systems which came into the scientific view in the last decades is the purinergic system.

Purinergic signalling

The first report on the extracellular effects of purines appeared in 1929 [193]. Previously, ATP was thought to have intracellular roles only. Afterwards, in the second half of the twentieth century, systematic research by Geoffrey Burnstock and other scientists revealed that extracellular purines are involved in virtually all body functions. Geoffrey Burnstock proposed the hypothesis of purinergic neurotransmission in the early 1970s [194], followed by a boom in this research area. As a result, we know that ATP is an important neurotransmitter and modulator in cell-to-cell communication in the central and peripheral nervous system, in neurons, neuron-to-glia or glia-to-glia communications [90, 195, 196].

A large chemical concentration gradient exists for ATP between the intra- and the extracellular space (millimolar vs. nano-/micromolar). Driven by this gradient, practically all cell types can be the source of extracellular ATP (in the CNS: neurons, glial cells, endothelium, vascular smooth muscle). The participation of vesicular release (as a transmitter or co-transmitter substance) and of membrane transport involving ATP-binding cassette proteins and permeation through hemichannels, plasmalemmal voltage-dependent anion channels and even P2X7 purinergic receptors operating as ATP-permeable channels, or osmotic transporters linked to anion channels have been described to increase extracellular ATP [197–199]. After their release from healthy cells, ATP and other purine and pyrimidine nucleotides and nucleosides fulfil mostly modulatory roles and are involved in both short- and long-term cellular communication [200, 201].

However, the peculiar nature of the purinergic system is that pathological concentrations of ATP may arise in the neighbourhood of injured or dying cells, mostly because of a spontaneous efflux of the purine via the damaged plasma membrane which is no longer a barrier for the extremely high intracellular ATP levels to spill out [202, 203].

Receptors for extracellular nucleotides have been designated as P2 receptors divided into two classes: the G protein-coupled P2Y receptors and the ligand-gated ion channel P2X receptors. They are further divided into subclasses P2Y₁, 2, 4, 6, 11–14, P2X₁–7), the expression profile of

which varies depending on the cell type. These receptors express specific agonist and antagonist selectivity for some natural and synthetic ligands (see comprehensive reviews of [204–206]).

Extracellular nucleotides can be rapidly hydrolyzed or interconverted by a complex family of ectoenzymes (e.g. ecto-ATPases, ecto-apyrases and ecto-5'-nucleotidases) which thereby either terminate their action or produce an active metabolite of altered receptor selectivity [207]. One of these products, adenosine, stimulates its own G protein-coupled P1 receptor class, the so-called adenosine (A1, A2A, A2B and A3) receptors [208].

P1 and P2 receptors are in many cases functionally antagonistic. Therefore, the breakdown of ATP cannot only limit its extracellular actions, but it balances the purinergic (e.g. P2/P1) signalling by bringing new players, with different properties, altered P2 and P1 receptor (subtype) selectivity into the game. Consequently, as it is illustrated in Fig. 1, purines represent a complex regulatory system with the involvement of the P2 receptors, the nucleotide hydrolyzing and interconverting enzymes and the P1 receptors [197].

P1 and P2 receptors show a widespread CNS distribution with both pre- and postsynaptic localizations [208]. Postsynaptic ionotropic P2X receptors may mediate fast synaptic currents, as it was first observed in the median habenula [209, 210], followed by reports about ATP-induced currents in other CNS regions, for example in the locus coeruleus [211], the hippocampus [212, 213] and the somatosensory cortex [214]. Nevertheless, excitatory postsynaptic currents (EPSCs) are largely mediated by ionotropic glutamate receptors, and a smaller component (5–15 % of total) may be due to the activation of P2X receptors [197, 215]. Noteworthy is that in the median habenula, EPSCs are solely ATPergic [197].

Besides P2X-mediated neurotransmission, nucleosides and nucleotides, via pre- and postsynaptic P1 and P2 receptors, can modulate the release or influence the postsynaptic effects of the major neurotransmitters [90, 202, 208, 216].

Purinergic modulation of glutamatergic neurotransmission

Adenosine mediates its neuromodulatory effects mostly via activating A1 and A2A receptors. In the vegetative nervous system, the G_i -coupled A1 receptor is inhibitory, while the preferentially G_s -coupled A2A receptor is excitatory at the presynaptic membranes [217–219]. Similarly, in the CNS, multiple neurochemical and electrophysiological evidence confirmed that A1 receptor activation reduced, and conversely, A2A activation facilitated glutamate release in various brain regions such as the cerebral cortex, striatum and hippocampus [218, 220, 221].

Regarding the P2 receptors, P2X receptors, possibly by elevating $[Ca^{2+}]_i$, facilitate neurotransmitter release. P2X-

mediated enhancement of glutamate release was observed in several regions of the CNS such as in the spinal cord, brain stem nuclei, locus coeruleus and hippocampus [90, 222–228]. In contrast, ATP has been reported to inhibit glutamate release by acting at metabotropic P2Y receptors for instance in the hippocampus and cortex [90, 227, 229]. Interestingly, the typically inhibitory presynaptic P2Y receptors are also implicated in potentiation of glutamate release in the median habenula nucleus [230].

In the rat hippocampus, ATP and its structural analogues which are rather resistant to enzymatic degradation inhibited glutamate release onto CA1 neurons via the activation of adenosine A1 receptors. It has been concluded, based on the use of selective antagonists and A1 receptor-deficient mice, that this inhibitory effect requires localized extracellular catabolism by ectonucleotidases and 'channelling' of the generated adenosine to A1 receptors [231, 232]. Similar data were reported also for the rat striatum [233] and medullary dorsal horn neurons [234].

The postsynaptic adenosine-NMDA interaction includes A1 receptor-mediated inhibition of NMDA currents, for instance in the hippocampus [235] or cerebral cortex [236]. Activation of a non-identified A2 receptor has been reported to potentiate AMPA receptors in the hippocampus [237]. In contrast, activation of the mostly stimulatory A2A receptors in the striatum inhibited rather than potentiated the NMDA currents in a subset of medium spiny interneurons [238, 239]. It is noteworthy that these striatal A2A receptors were coupled to G_q rather than G_s and to the activation of phospholipase C/inositol trisphosphate/calmodulin and CaMKII pathway [238].

Extra-striatal A2A receptors control both the release of glutamate and postsynaptic NMDA receptors in a facilitatory manner [240]. This is in contradiction with the previously reported findings that such receptors are inhibitory in a subpopulation of striatal medium spiny (probably striatopallidal) output neurons [238, 239]. However, A2A receptors at striatal medium spiny neurons appear to be an 'aberrant' population; they display properties often opposite to those displayed by other A2A receptors [241, 242].

In the hippocampus, mGlu5 receptors (a subtype of the group I metabotropic glutamate receptors) enhance NMDA-mediated effects [243]. It has been shown by recording field excitatory postsynaptic potentials (fEPSPs) from the CA1 area that an endogenous A2A receptor-mediated tone was required to enable mGlu5 receptors to potentiate NMDA effects [244, 245]. The authors forwarded the hypothesis that hippocampal A2A and mGluR receptors are co-located and act synergistically.

While in the previous studies the origin of adenosine (neuronal or astrocytic) exerting tonic activation of NMDA receptors was not clarified, the use of astrocyte-specific inducible transgenic (dnSNARE) mice confirmed that synaptic NMDA

Fig. 1 The purinergic system. 1 ATP is released from healthy cells into the extracellular space by vesicular release (as a transmitter or co-transmitter substance) or via ion channels/hemichannels/transporters. 2 Spontaneous efflux of purines occurs from injured or dying cells resulting in pathological concentrations of ATP in the extracellular space. 3 Ectoenzymes rapidly hydrolyze or interconvert the extracellular nucleotides thereby either terminating their action or producing an active metabolite of altered receptor selectivity. 4

Postsynaptic ionotropic P2X and metabotropic P2Y receptors mediate fast and slow synaptic responses, respectively. 5 Extracellular purines activate pre- and postsynaptic P2X and P2Y receptors; adenosine stimulates its own P1 receptor class. These receptors (further divided into subclasses) modulate the effects of classic neurotransmitters (e.g. glutamate acting at its ionotropic receptors); thereby, purines represent a complex neuromodulatory system involved in fine-tuning of neurotransmission

currents in layer 2/3 pyramidal neurons of the somatosensory cortex are diminished in such animals [246]. Further, astrocytic dnSNARE expression led to decreased A1 receptor-mediated tyrosine phosphorylation of Src kinase and less surface expression of NR2B subunits as well as a smaller NMDA component of miniature EPSCs [247].

Adenosine is a potent anticonvulsant acting on excitatory synapses through A1 receptors [248]. However, the observed synaptic depression is not a consequence of the release and consecutive degradation of astrocytic ATP, but instead an autonomic feedback mechanism that suppresses excitatory transmission during prolonged activity [249]. Thus, adenosine is released due to the strong stimulation of neuronal postsynaptic NMDA receptors and subsequently acts via inhibitory neuronal A1 receptors.

In the paraventricular nucleus of the hypothalamus, ATP of glial origin potentiated glutamatergic EPSCs through the stimulation of postsynaptic P2X7 receptors [250]. Specific interaction between P2X and NMDA receptors has not yet been reported at the postsynaptic level. However, Ca^{2+} entry via P2X receptors may cause modulation of NMDA receptor function, for example by calcium-dependent inactivation [251]. Further, Baxter et al. [252] reported that the absence of P2X4 receptors limited the incorporation of GluN2B subunits into synaptic NMDA receptors in mouse hippocampus.

In the prefrontal cortex, a complex interaction has been revealed involving multiple glial and neuronal P2Y receptor

subtypes as well as metabotropic and ionotropic glutamate receptors of NMDA subtype located on layer V pyramidal cells. This interaction will be discussed in the following chapter.

Interplay between purinergic and glutamatergic systems in the prefrontal cortex

The medial PFC is crucially involved in higher order cognitive functions, such as attention, memory and learning, problem solving, planning and orchestration of thoughts and actions in accordance with internal goals [253, 254]. Its dysfunctions may contribute to the pathogenesis of schizophrenia, addiction and cognitive disorders [255]. A complex interplay has been elucidated in layer V pyramidal cells of the prefrontal cortex between purinergic and glutamatergic systems, raising the possibility that purinergic neuromodulation is involved in the regulation of cognitive functions, as it was later confirmed by *in vivo* behavioural pharmacology tests.

Layer V pyramidal neurons are the key elements of the PFC, and they are crucial in the network activity underlying working memory, a type of short-term memory necessary for goal-directed behavioural sequences and decision-making [256]. These neurons receive afferent innervation from the dopaminergic cell groups localized in the ventral tegmental area (VTA), being critical for the modulation of cognitive functions [257]. Glutamatergic input to PFC neurons

originates from the mediodorsal nucleus of the thalamus and from neighbouring PFC pyramidal cells interconnected with each other [258]. Dopaminergic and glutamatergic input converges on layer V pyramidal cells [259]. Prefrontal glutamatergic projections innervate the VTA (dopamine neurons that project back to the PFC) and control dopaminergic neuronal activity in the nucleus accumbens involving neurons in the pedunculo-pontine tegmentum and the laterodorsal tegmentum [260, 261].

Early observations raised the possibility that the purinergic and glutamatergic systems may interact at the level of layer V pyramidal neurons in the PFC. Especially the dopaminergic modulation of NMDA receptors, mentioned above [115, 116, 118, 119], and the recognition of ATP as a co-transmitter substance, particularly its co-release with catecholamines [262–264], strengthened the idea that the possible co-transmitter of dopamine, ATP, may shape the glutamatergic excitation in the prefrontal cortex. Indeed, P2Y receptors were shown to positively interact with NMDA receptors located on layer V pyramidal neurons of rat PFC [265], just as dopamine does [116]. However, an inhibitory interaction between P2Y and NMDA receptors was also reported by the same group. This latter interaction was mediated by the P2Y1 subtype of the metabotropic ATP receptors and persisted when voltage-gated Na^+ channels or G protein-mediated signalling was blocked. Therefore, a membrane-delimited cross-talk between P2Y1 and NMDA receptors has been suggested to operate in pyramidal neurons [266]. Subsequently, the characteristics of the facilitatory interaction were further analysed. Especially, the acknowledgement of glia as an active partner in synapses and its possible involvement in cellular circuitries for synaptic plasticity [74] as well as the reports on the role of ATP in neuron-glia communication [88–90, 201] fertilized this research.

Reinvestigation of the facilitatory P2Y-NMDA interaction suggested that ATP activated astrocytic P2Y4 receptors and thereby released vesicular glutamate onto neighbouring neurons. This glutamate stimulated type I mGlu receptors which potentiated NMDA currents through the G_q /phospholipase C/inositol trisphosphate/ Ca^{2+} /CaMKII transduction pathway.

Interestingly, subsequent experiments showed that in contrast to the exocytotic glutamate release by P2Y4 receptors, which resulted in activation of group I mGlu receptors, glutamate accumulating after blockade of astrocytic glutamate uptake leads to the stimulation of group II mGlu receptors. Either group of mGlu receptors interacted with NMDA receptors in a facilitatory manner [104]. In perfect agreement with the previous observations in acutely dissociated pyramidal neurons of the prefrontal cortex, ATP depressed the NMDA current amplitudes [105].

Eventually, P2Y1 receptors have also been reported to inhibit long-term depression in PFC and to modulate synaptic plasticity, probably reducing Ca^{2+} transients associated with postsynaptic

voltage-sensitive Ca^{2+} channels on layer V pyramidal neurons [267, 268]. Figure 2 illustrates the modulation of NMDA receptors in pyramidal cells of PFC by purinergic receptors.

In view of the observed involvement of glia in prefrontal P2Y-NMDA interaction, it is noteworthy that in rat hippocampal slices activation of astroglial P2X7 receptors resulted in glutamate release and subsequent activation of neuronal currents [269]. However, in the rat PFC, P2X7 receptor activation failed to enhance EPSCs in layer V pyramidal cells evoked by electrical stimulation in layer I/II [270].

Data obtained from in vitro experiments gain more practical importance if these results can be converted to effects under in vivo experiments. Memory retention was reported to be sensitive to P2 receptors blockade in young animals [271]. A recent publication analysed behavioural effects of MRS2365, a selective P2Y1 receptor agonist administered locally into the PFC. The experiments revealed that activation of P2Y1 receptors in the PFC impaired inhibitory control and behavioural flexibility mediated by increased mesocorticolimbic activity and local disinhibition [272]. In rats, the spatial delayed win-shift task showed that prefrontal P2Y1 receptors were not primarily involved in the ‘short-term’ storage of information. However, P2Y1 receptor activation in the PFC influenced its processing during and after a delay (‘recall’). Thus, the pre-trial infusion of the selective P2Y1 agonist MRS2365 caused more errors in the phase after the delay than that of vehicle [273]. In a social discrimination setting, where an adult animal has to distinguish between a familiar and unfamiliar juvenile, MRS2365 applied into the rat PFC diminished the naturally preferred investigation of the unfamiliar juvenile [273]. In mice, stress-induced depressive-like behaviour was investigated, and the stimulation of endogenous ATP release from astrocytes induced antidepressive-like effects, mediated by P2X2 receptors located in the PFC. Infusion of purinergic agonists into the PFC but not into the hippocampus reversed the stress-induced behavioural changes [274].

Considering that cognitive alterations in attention, behavioural inhibition, learning and memory identified in addictive disorders are strongly related to dysfunctions of the PFC, the involvement of P2 receptors is highly probable in the pathophysiology of addiction [275]. Stimulants, like amphetamine, release dopamine in the PFC by the activation of dopaminergic cell bodies in the nucleus accumbens, which in turn results in the stimulation of glutamatergic projections to the nucleus accumbens and ventral tegmental area [276, 277]. Application of P2 receptor agonists in the nucleus accumbens of rats raised the extracellular level of dopamine [278], accompanied with enhanced locomotion [279].

Repeated exposure to psychostimulants such as amphetamine causes an enhancement of the motor stimulant effect elicited by a subsequent drug challenge (‘behavioural sensitization’) [280]. Repetitive stimulation of

Fig. 2 Modulation of layer V pyramidal cells of the prefrontal cortex (PFC) by purinergic receptors. ATP exerts dual modulatory effects on NMDA receptors located in layer V pyramidal cells of rat PFC. (1) On the one hand, a positive interaction involves neuron-glia communication with a complex interplay of ATP and glutamate. By activating astrocytic P2Y4 receptors, ATP induces vesicular glutamate release from glial cells onto neighbouring neurons. This glutamate stimulates type I mGluR receptors located on layer V pyramidal neurons, which in turn potentiates NMDA currents through the G_q /phospholipase C/inositol trisphosphate/ Ca^{2+} /CaMKII transduction pathway. (2) On the other hand, a negative interaction also takes place between purinergic and NMDA receptors in the prefrontal cortex. ATP activating P2Y1 receptors located in layer V pyramidal cells (in close proximity to the NMDA receptors), by a membrane-delimited cross-talk between the receptors, inhibits the NMDA receptor channels

ventral tegmental dopaminergic neurons, endowed with P2Y1 receptors, by 2-MeSATP induced behavioural sensitization to a single amphetamine challenge [281]. Furthermore, enhanced P2Y1 receptor immunoreactivity was observed in the brain after sensitization with amphetamine [282]. Intracerebroventricular pre-treatment with the P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2', 4'-disulfonic acid (PPADS) prevented both the acute locomotor effects of amphetamine and the behavioural sensitization caused by repeated amphetamine injections in rats [283]. It was suggested that the activation of P2 (probably P2Y1) receptors by endogenous ATP was an intermediary step in the sensitization process to amphetamine.

Purinergic mechanisms set the stage for synaptic plasticity via interaction with the glutamate system: learning and memory

Long-term and bidirectional changes in synaptic strength are thought to provide a cellular basis for information storage in neuronal networks [284]. LTP and LTD mean marked increase and decrease of evoked synaptic currents, respectively, after delivery of high-frequency stimulation to the neuronal inputs. The facilitation or depression of synaptic activity may persist for hours and occur throughout the mammalian brain. Different areas in the brain exhibit different forms of LTP, but even

in the same area, e.g. the hippocampus, this phenomenon relies on the pathways stimulated (Schaffer collaterals, NMDA-dependent; mossy fibres, NMDA-independent) [285]. Tetanic stimulation of presynaptic neurons often induces LTP, while low-frequency stimulation (LFS) induces LTD [286]. Furthermore, LTP induction in one pathway could be accompanied by heterosynaptic LTD in adjacent unstimulated pathways, providing a mechanism for local sharpening of activity-induced synaptic potentiation [287].

There are a number of excellent reviews available on plasticity changes by adenosine A1 and especially A2A receptors [217, 288–290], and therefore, we will put emphasis on the relatively scarce specific information related to a similar function of P2X/P2Y receptors.

A large amount of data exists to indicate that adenosine fine-tunes synaptic transmission over a wide spatial and temporal scale, consistent with its well-established ability to facilitate or restrict plasticity at different synapses [288, 290]. A1 receptor activation inhibits NMDA-mediated currents in hippocampal [235] and cortical neurons [236] and thereby might interfere with synaptic plasticity. A2A receptors regulate plasticity in the hippocampus by their co-localization and synergistic action with metabotropic glutamate receptors facilitating NMDA-mediated currents [244, 245]. Moreover, the ability of A2A receptors to enhance the activity-dependent efficiency of excitatory synapses has been argued to result from a number of additional mechanisms, such as (i) enhanced release of neurotransmitters [291], (ii) facilitation of brain-derived neurotrophic factor-induced signalling [292, 293], (iii) enhanced responsiveness of NMDA receptors to transmitter glutamate [294, 295] and (iv) increased availability of GluR1-containing AMPA receptors for synaptic insertion [296].

A hitherto missing link between hippocampal CA3–CA1 LTP and associative learning was established by *in vivo* experiments on conscious behaving mice [297]. The highly selective A2A receptor antagonist 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH58261) interfered both with the LTP induced by stimulation of Schaffer collateral-commissural fibres as well as with an associative learning paradigm (classical eyeblink conditioning). It was concluded that the endogenous activation of A2A receptors plays a pivotal effect in the associative learning process and its relevant hippocampal circuits, including activity-dependent changes at the CA3–CA1 synapse.

With respect to the above findings, it is quite unexpected that the combined A1/A2A receptor antagonistic caffeine causes beneficial effects in animal models of conditions expected to impair memory performance such as Parkinson's disease, chronic stress, type 2 diabetes, attention deficit and hyperactivity disorder, early life convulsions or alcohol-induced amnesia [298, 299] (see also “Participation of

purinergic mechanisms in neurological and psychiatric illnesses: possible therapeutic perspectives”). This contradiction still needs further explanation, but the following points should be certainly considered: (1) Caffeine is, in addition to its A1/A2A receptor antagonistic properties, also a blocker of phosphodiesterases. Phosphodiesterase inhibition is supposed to be a new mechanism of cognition enhancement [300]. (2) Caffeine is neuroprotective in all types of neurodegenerative diseases, due to the blockade of A1/A2A receptors [298, 301]. This drug appears to act only mildly in young rats, but it prevents the cognitive decline associated with ageing [302] or neurodegeneration (Alzheimer’s disease [303], Huntington’s disease [304]). (3) A2A receptor antagonists, including caffeine, can exert the same allosteric modulation of D2 ligands than A2A receptor agonists, while A2A receptor agonists and antagonists counteract each other’s effects [305]. A similar allosteric interaction might take place between A2A/NMDA receptors. The combined effects at these four targets could explain the ‘aberrant’ actions of caffeine.

Inhibition of P2X receptors on hippocampal CA1 pyramidal neurons by the non-selective P2X/Y receptor antagonist PPADS dramatically facilitated the induction of LTP [251]. It can be assumed that P2X1 or P2X3 receptors are involved, because a similar potentiation of LTP resulted after desensitization of α, β -methylene ATP currents. In view of the well-known Ca^{2+} permeabilities of P2X and NMDA receptors and the failure of PPADS to act after pharmacological blockade of NMDA receptors, it was hypothesized that P2X receptors affect the threshold for LTP by altering Ca^{2+} -dependent inactivation of NMDA receptors. A recent study revealed that stimulation of postsynaptic P2X2 receptors decreased the amplitude of miniature EPSCs and AMPA-evoked currents in cultured hippocampal neurons, probably promoting clathrin-mediated endocytosis of postsynaptic AMPA receptors. Notably, endogenous ATP of glial origin participated in this interaction [306]. Considering that synaptic strength is regulated by the balance between the insertion and internalization of AMPA receptors into the postsynaptic membrane, this is one of the possible mechanisms by which P2X receptors affect synaptic plasticity at glutamatergic synapses [307–309].

Heterosynaptic (h)LTD at untetranized synapses was shown to accompany the induction of LTP in the hippocampal CA1 region. It has been shown that ATP released by astrocytes and activating P2Y receptors located at neighbouring nerve terminals caused hLTD; P2Y receptor occupation may depress glutamate release under these conditions [310]. Selective stimulation of astrocytes expressing channel rhodopsin-2, a light-gated cation channel permeable to Ca^{2+} , resulted in LTD of synapses on neighbouring neurons. This synaptic modification required Ca^{2+} elevation in astrocytes and activation of P2Y but not NMDA receptors. It is interesting to note that astrocytic ATP after degradation to adenosine also mediated activity-dependent heterosynaptic depression [85]. This effect

was due to the stimulation of A1 receptors as proven by the use of selective agonists and antagonists.

We mentioned already in the previous chapter that P2Y1 receptor activation inhibits LTD in the prefrontal cortex. This phenomenon may be a correlate of the impaired cognition in various behavioural tests after stimulation of P2Y1 receptors in the rat medial prefrontal cortex [272].

Participation of purinergic mechanisms in neurological and psychiatric illnesses: possible therapeutic perspectives

Once again, the availability of many comprehensive review articles on the involvement of A1/A2A [240, 241, 298] and P2X/Y receptor [202, 311–313] in the pathophysiology of neurological and psychiatric illnesses allows us to concentrate on a few items of particular relevance for purine/glutamate interaction in these diseases. Actually, the modulation of purinergic mechanisms may improve mood disorders, depression, schizophrenia, attention deficit/hyperactivity disorder and all types of neurodegenerative illnesses, which will not be discussed in an exhaustive manner.

The present hypotheses about the etiology of schizophrenia include both a hyperfunction of the mesocorticolimbic dopamine system and a hypofunction of the glutamatergic afferents from the prefrontal cortex to the ventral tegmental area [314]. Adenosine may interact with these systems both via A1 and A2A receptors [315]. Of eminent significance appear to be the functionally antagonistic A2A/D2 and A2A/NMDA interactions at striatopallidal GABAergic neurons, but the A1 receptor-mediated inhibition of glutamate release [316] and the postsynaptic depression of NMDA receptors [235] may be also relevant. Based on these observations, the adenosine system should be simultaneously targeted both at its A1 and A2A receptor-modulated arms for possible therapeutic purposes [315].

Recently, it has been reported that several non-pharmacological treatments of depression increase adenosine concentration and/or upregulate A1 receptors in the brain [317]. Upregulating A1 receptors in transgenic mice led to pronounced acute and chronic resilience towards depressive-like behaviours in various tests. Conversely, A1 receptor-deleted mice displayed an increased depressive-like behaviour and were resistant to the antidepressant effects of sleep deprivation. These results contradict older data, which show that adenosine agonists and drugs which increase adenosine bioavailability, such as adenosine deaminase inhibitors, mimic depressive behaviour [318] and adenosine antagonists such as caffeine (A1/A2A) and SCH412348 (only A2A) have clear antidepressant effects in behavioural paradigms [319, 320]. One of the reasons for the apparent discrepancy between the older and newer results may be that A2A receptors modify A1 receptor-mediated effects. The common transduction pathway of A1/A2A receptors might be NMDA receptors, whose

blockade, e.g. by ketamine, has rapid and potent antidepressive effects in clinically manifest and treatment-resistant major depression and bipolar depression [321, 322].

Not only adenosine but also ATP participated in the induction of depression-like behaviour in mice. This ATP was of astroglial origin and was shown to activate P2X2 receptors in the medial prefrontal cortex [274]. In good correlation with this finding, post-mortem brain analyses and imaging studies of patients with depression have implicated glial dysfunction in the pathology of major depressive disorder.

The motor symptoms of Parkinson's disease (PD) are primarily due to the degeneration of the dopaminergic neurons in the nigrostriatal pathway. Compelling evidence suggests that antagonists of the A2A receptor successfully alleviate symptoms in PD patients [323]. Rodent studies support the hypothesis that the therapeutic effects of A2A receptor antagonists are achieved via a reduction of the inhibitory output of the basal ganglia indirect pathway. The hypothesized neuroprotective mechanisms include inhibition of the presynaptic A2A receptor-mediated glutamate release from neurons, and of the postsynaptic negative interaction between A2A and D2 or NMDA receptors at striatopallidal output neurons [324, 325].

In animal models of Huntington's disease (HD), changes in NMDA receptor expression and increased sensitivity to NMDA-induced toxicity have been demonstrated [326]. Blockade of A2A receptors resulted in neuroprotection in a rat model of HD [327]. In this respect, it was interesting to observe that chronic A2A receptor blockade in HD mice remodelled NMDA receptors in the striatum by modifying NR1 and NR2A/NR2B expression [328].

In addition to these examples of neurodegenerative illnesses, where glutamate/adenosine interaction is most prominent, secondary degeneration of neurons and glial cells in CNS trauma, ischemia, stroke, Alzheimer's disease, PD, HD, multiple sclerosis, amyotrophic lateral sclerosis and epilepsy occurs due to P2X7 receptor activation because of the massive release of ATP from the damaged brain or spinal cord tissue [202, 329, 330]. On the one hand, P2X7 receptors localized at the nerve terminals themselves or at astrocytes signalling to these terminals may greatly increase the release of glutamate, and on the other hand, P2X7 receptors can directly damage neurons and glial cells [331, 332]. These receptors are permeable for Ca^{2+} ions; their activation largely increases intracellular Ca^{2+} and thereby initiates apoptotic/necrotic mechanisms which eventually lead to neuronal/glial death. All neurodegenerative illnesses are accompanied by cellular damage in the neighbourhood of the immediately afflicted area. This secondary degeneration may be prevented/ameliorated by P2X7 receptor antagonists [329]. In addition, multiple interactions of P2X7 receptors with postsynaptic glutamate effects have to be also considered.

Outlook

The tightly controlled modulation of glutamatergic transmission is of enormous importance in brain physiology and in pathogenesis of CNS illnesses including psychiatric dysfunctions. Here we conclude based on published evidence that besides the well-known, classical modulators such as dopamine or acetylcholine, the purinergic neuromodulatory system is also fundamentally involved in the fine-tuning of glutamatergic transmission.

There are a number of open or ambiguous questions relating to the interaction of the purinergic and glutamatergic systems. They are mostly due to the heterogeneous effects caused by ATP and its enzymatic degradation product adenosine on the release and postsynaptic function of glutamate. For example, ATP itself might increase glutamate release via P2X7 receptors, or after decomposition to adenosine, it might either increase (via A2A) or inhibit (via A1 receptors) the release of glutamate. Similarly, A1 receptors inhibit and A2A receptors facilitate NMDA receptor-mediated effects. Moreover, A2A receptors can differentially influence NMDA receptors depending on the brain area investigated (stimulation in the hippocampus, but inhibition in the striatum). The situation becomes still more complicated because of the presence of ATP and adenosine sensitive receptors at different cell types (neurons, macro- and microglia, ependymal and vascular cells) and the temporal diversity of the onset and offset of their activation. For example, after the occlusion of a cerebral artery, P2X7 receptors are upregulated soon after the ensuing metabolic limitation, and P2X7 receptor upregulation occurs only with some temporal delay on astrocytes and oligodendrocytes. Eventually, both ATP and adenosine are polar compounds, which do not pass the blood-brain barrier and therefore cannot be used on systemic application.

In consequence, for possible therapeutic purposes, blood-brain barrier permeable and enzymatically stable substances should be used to directly stimulate or inhibit P2/P1 receptors; an alternative possibility is to interfere with the inactivation of ATP/adenosine (uptake or enzymatic degradation) or to modulate their effects by allosteric agonists/antagonists. The greatest chances to become drugs have substances which antagonize P2X7 or A2A receptors. P2X7 receptors have a low density in the healthy brain and seem to be upregulated only during pathophysiological conditions; therefore, their blockade is expected to have only moderate side effects by leaving physiological functions unaltered. The same holds true for striatal A2A receptors, which clearly differ in their properties from their extra-striatal counterparts and might be quite selectively targeted for the therapy of, e.g. Parkinson's disease.

It has to be stated that not all effects of purinergic ligands can be predicted with a high likelihood. The mild psychostimulant caffeine, which has been 'abused' for

centuries, exerts this effect by blocking A2A receptors in the nucleus accumbens but, in addition, acts as a cognition enhancer and improves neurodegeneration by a presently unclarified mechanism. Thus, structural analogues of caffeine have good chances to become suitable drugs for the treatment of these latter disorders.

In conclusion, the understanding of purinergic/glutamatergic interaction in the brain may open new avenues in the target identification and optimization of research activities aiming at the restoration of glutamatergic neurotransmission in pathological states.

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