Is the functional interaction between adenosine A\textsubscript{2A} receptors and metabotropic glutamate 5 receptors a general mechanism in the brain? Differences and similarities between the striatum and the hippocampus

M. T. Tebano· A. Martire · R. Pepponi · M. R. Domenici · P. Popoli

Abstract The aim of the present paper was to examine, in a comparative way, the occurrence and the mechanisms of the interactions between adenosine A\textsubscript{2A} receptors (A\textsubscript{2ARs}) and metabotropic glutamate 5 receptors (mGlu5Rs) in the hippocampus and the striatum. In rat hippocampal and corticostriatal slices, combined ineffective doses of the mGlu5R agonist 2-chloro-5-hydroxyphenylglycine (CHPG) and the A\textsubscript{2AR} agonist CGS 21680 synergistically reduced the slope of excitatory postsynaptic field potentials (fEPSPs) recorded in CA1 and the amplitude of field potentials (FPs) recorded in the dorsomedial striatum. The cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway appeared to be involved in the effects of CGS 21680 in corticostriatal but not in hippocampal slices. Interestingly, the A\textsubscript{2AR} antagonist ZM 241385 significantly reduced the NMDA-potentiating effect of CHPG. In primary cultures of rat hippocampal and striatal neurons (ED 17, DIV 14), CHPG significantly potentiated NMDA-induced lactate dehydrogenase (LDH) release. Again, such an effect was prevented by ZM 241385. Our results show that A\textsubscript{2A} and mGlu5 receptors functionally interact both in the hippocampus and in the striatum, even though different mechanisms seem to be involved in the two areas. The ability of A\textsubscript{2ARs} to control mGlu5R-dependent effects may thus be a general feature of A\textsubscript{2ARs} in different brain regions (irrespective of their density) and may represent an additional target for the development of therapeutic strategies against neurological disorders.

Key words adenosine A\textsubscript{2A} receptors · extracellular recording · hippocampus · lactate dehydrogenase (LDH) · metabotropic glutamate 5 receptors (mGlu5Rs) · neuronal cultures · NMDA · protein kinase A · striatum

Introduction

Adenosine A\textsubscript{2A} receptors (A\textsubscript{2ARs}) are the most abundant adenosine receptor subtypes in the striatum, where they play a prominent role in modulating glutamatergic input to striatal GABAergic neurons. These receptors are almost exclusively localized in the striopallidal neurons, which also show a high degree of expression of metabotropic glutamate 5 receptors (mGlu5Rs) [1]. It has been previously demonstrated that mGlu5Rs are particularly involved in the modulation of striatal functions such as motor activity [2] and synaptic plasticity [3] and that they play a role in neurodegenerative processes [4]. Functional interactions between striatal mGlu5Rs and A\textsubscript{2ARs} have been reported in
the modulation of several effects, such as motor behavior in models of Parkinson’s disease [2, 5, 6], γ-aminobutyric acid (GABA) and glutamate release [7, 8], c-fos expression [9], and DARPP-32 phosphorylation [10]. Such functional interactions were supported by the demonstration of the existence of heteromeric complexes containing A2A and mGlu5 receptors in striatal neurons [9].

Although adenosine A2A receptors are mostly expressed in the striatum, a limited degree of expression also exists in the hippocampus, where such receptors exert an excitatory influence on normal synaptic transmission and modulate excitotoxic processes [11]. Like the striatum, the hippocampus shows a marked expression of mGlu5Rs (mainly located in the CA1 area), which, by setting the tone of N-methyl-D-aspartate (NMDA) receptors [12–14], mediate several physiological and pathological processes such as learning and memory [15, 16], generation of epileptic activity [17], and excitotoxicity [18]. Very recently, we have demonstrated that A2A and mGlu5Rs are colocalized in the rat hippocampus [19].

Figure 1 Adenosine A2A activation facilitates CHPG-induced effects in hippocampal and corticostriatal slices. Superfusion of rat hippocampal and corticostriatal slices with the selective mGlu5R agonist CHPG (1 mM) induced a comparable decrease of the fEPSP slope and FP amplitude that recovered after washing (a). The co-application of concentrations of CGS 21680 (50 nM) and CHPG (500–750 μM), which were ineffective by themselves, significantly reduced the fEPSP slope in hippocampal slices and the FP amplitude in corticostriatal slices (b). The graphs represent the average time course of changes in the fEPSP slope (N = 6–13 experiments) and FP amplitude (N = 10–15 experiments). All the values are expressed as percentage of baseline values (mean ± SEM). The period of drug application is indicated by the *horizontal bars*.

The activation of A2A receptors facilitates mGlu5R-mediated responses in both striatal and hippocampal preparations.

Electrophysiological experiments were performed in both corticostriatal and hippocampal slices from rats as previously described [19, 20]. Extracellular field excitatory postsynaptic potentials (fEPSPs) and extracellular field potentials (FPs) were recorded in the stratum radiatum of the CA1 area or in the dorsomedial striatum. To allow comparisons between different experiments, in each experiment the values of the fEPSPs slope and of the FP amplitude were normalized, taking as 100% the average of the control values. The selective mGlu5R agonist 2-chloro-5-hydroxyphenylglycine (CHPG) did not affect the electrical responses of hippocampal and striatal neurons at μM concentrations. As shown in Figure 1a, only at concentrations of 1 mM did CHPG induce a reduction of the fEPSPs slope in the hippocampus (-21.8 ± 4% of basal) and of the FP amplitude in the striatum (-15.7 ± 5.4%); in both cases, CHPG-induced reductions completely...
recovered after washout. The effect of CHPG was significantly reduced by the selective mGlu5R antagonist MPEP (30–50 μM) in both the hippocampus and the striatum (MPEP + CHPG: -7.1 ± 1.5% of basal, P < 0.01 vs CHPG alone in the hippocampus; MPEP + CHPG: -3.53 ± 0.44% of basal, P < 0.05 vs CHPG alone in the striatum).

To investigate whether the activation of A2ARs could have a facilitatory role on mGlu5R-mediated effects, CGS 21680, a selective A2AR agonist, was used. Combined ineffective doses of CHPG and CGS 21680 (500 μM + 50 nM in the hippocampus; 750 μM + 30 nM in the striatum) synergistically reduced the slope of fEPSPs recorded in CA1 (-25.0 ± 5.4%, P < 0.005 vs basal) and the amplitude of field potentials (FPs) recorded in the dorsomedial striatum (-13.4 ± 2.7%, P < 0.05 vs basal), whereas neither CGS 21680 nor CHPG affected synaptic transmission on their own (Figure 1b). Both MPEP (30 μM) and the selective A2AR antagonist ZM 241385 (100 nM) abolished the synergistic effect resulting from the coactivation of A2A and mGlu5Rs (not shown).

Different mechanisms are involved in the A2A/mGlu5 interaction in the two brain areas

In order to explore the mechanisms underlying the facilitatory role of CGS 21680 towards CHPG, the influence of the adenylate cyclase activator forskolin and of the protein kinase A (PKA) inhibitor KT5720 were studied. Interestingly, the potentiating effect of CGS 21680 towards CHPG was reproduced by forskolin and abolished by KT 5720 in corticostriatal but not in hippocampal slices. Co-application for 10 min of forskolin (100 nM) and CHPG induced a transient depression of the response (-17.99 ± 4.89% with respect to basal) in corticostriatal slices (Figure 2a,b) Conversely, forskolin (up to 30 μM) failed to potentiate CHPG responses in the hippocampus. Furthermore, the synergistic depression induced by CHPG plus CGS 21680 was prevented by the PKA inhibitor KT 5720 (4 μM) only in the striatum. These results indicate an involvement of the cyclic adenosine monophosphate (cAMP)/PKA pathway in the A2A/mGlu5 interaction in the striatum but not in the hippocampus.

A postsynaptic locus of interaction is more likely in both the striatum and the hippocampus

Since both A2A and mGlu5Rs modulate glutamate release [21–23], we wanted to establish the possible involvement of presynaptic mechanisms in the effects of A2A and mGlu5 receptor agonists. To this end, a series of experiments was performed under a protocol of paired pulse stimulation (PPS), in which the afferents fibers were stimulated twice with an interpulse interval of 50 ms. In control conditions, such a protocol normally elicits a condition of paired pulse facilitation (PPF), in which the response elicited by the second stimulus (R2) is greater than the response elicited by the first stimulus (R1). Modification of this phenomenon is usually attributed to a presynaptic calcium-dependent change in release probability at both excitatory and inhibitory synapses. When PPF is increased by a drug, it suggests an inhibition of release probability and when PPF is decreased, up to a change in paired pulse depression (PPD), it suggests a facilitation of release probability [24]. The degree of PPF is quantified by the R2/R1 ratio. The application of CGS 21680 and/or CHPG did not modify the
R2/R1 ratio during the PPS protocol \( (P > 0.05 \text{ vs control in both areas, data not shown}) \). Thus, although a lack of effect upon paired pulse stimulation does not allow to definitely exclude a presynaptic locus of action, such a possibility appears not very likely.

In both brain areas, the state of activation of A2A receptor influences the ability of mGlu5Rs to potentiate NMDA effects.

Slice perfusion for 10 min with NMDA (8 and 12.5 \( \mu \text{M} \)) induced a comparable reduction of the fEPSP slope (\( \cong 27\% \)) and of the FP amplitude (\( \cong 23\% \)) in hippocampal and corticostriatal slices, respectively. Such an effect was strongly potentiated by the application of CHPG 500–750 \( \mu \text{M} \).

![Graph](image-url)

**Figure 3** CHPG-potentiated NMDA effects in hippocampal and corticostriatal slices. Graphs represent the time course of changes in FP amplitude and fEPSP slope in two typical experiments recorded in corticostriatal (a) and in hippocampal slices (b), respectively. A 10-min superfusion period with NMDA (12.5 and 8 \( \mu \text{M} \)) reduced the synaptic response, an effect that was potentiated by the co-application, 30 min later, of 750 \( \mu \text{M} \) (a) or 500 \( \mu \text{M} \) (b) CHPG. Each point represents the means of three responses evoked by stimulation of afferents fibers in the two areas.

![Graph](image-url)

**Figure 4** The blockade of either A2A or mGlu5 receptors prevents the ability of CHPG to potentiate NMDA effects in hippocampal and corticostriatal slices. The histograms show that ineffective concentrations of CHPG (500–750 \( \mu \text{M} \)) significantly potentiated NMDA-induced reduction in FP amplitude (a) or fEPSP slope (b) and that both the mGlu5R antagonist MPEP (30 \( \mu \text{M} \)) and the A2A receptor antagonist ZM241385 (30–100 nM) prevented the effects of CHPG. All values are expressed as percentage of the NMDA effects and represent the mean \( \pm \) SEM values from 4–6 experiments in each group. \*P < 0.05 (Mann-Whitney U test) vs NMDA alone; \#P < 0.05 (Mann-Whitney U test) vs CHP + NMDA.

\( 286.6 \pm 16.9\% \) and \( 329.97 \pm 38.68\% \) of NMDA alone in the hippocampus and in the striatum, respectively; \( P < 0.05 \) in both cases) (Figure 3a,b). In the hippocampus, the NMDA-potentiating effect of CHPG was completely blocked by the selective mGlu5R antagonist MPEP (30 \( \mu \text{M} \)) and significantly attenuated by the A2A receptor antagonist ZM 241385 (30–100 nM) (Figure 4a). When given alone, neither MPEP nor ZM 241385 influenced the basal synaptic transmission or the NMDA-induced inhibition of the fEPSP slope (not shown). In the striatum, not only MPEP (30 \( \mu \text{M} \)) but also ZM...
A tone at $A_{2A}$Rs is required to allow mGlu5Rs to potentiate NMDA toxicity.

To evaluate whether the $A_{2A}$/mGlu5 receptor interaction also played a role in the modulation of NMDA-induced toxicity, primary cultures of rat striatal and hippocampal neurons were used. Neurons isolated from 17-day-old rat embryos, plated in Neurobasal medium and grown in vitro for 13–15 days, were used. Neuronal cell injury was quantified by the measurement of lactate dehydrogenase (LDH) released in the culture medium. Incubation for 1 h of neuronal cultures with 300 µM NMDA induced a comparable increase of LDH release in hippocampal (190.2 ± 32.4%) and striatal cell cultures (170.3 ± 18.46). CHPG 1 mM (ineffective by itself) significantly potentiated NMDA-induced LDH release (312.4 ± 60.1% in the hippocampus; 249.5 ± 31.64% in the striatum; $P < 0.05$ vs NMDA alone). The potentiating effect of CHPG on NMDA-induced LDH release was prevented by both the $A_{2A}$ and mGlu5 receptor antagonists. In fact, 15 min of pretreatment with MPEP (30 µM) or ZM 241385 (30 nM) were able to restore the same levels of LDH induced by NMDA alone ($P < 0.05$ vs CHPG ± NMDA). When applied alone, neither MPEP nor ZM 241385 influenced basal or NMDA-induced LDH release (Figure 5).

Discussion

The main finding of this paper is that the mGlu5Rs, in the two brain areas where they show the highest levels of expression (namely, the striatum and the hippocampus), are under the tight control of the adenosine $A_{2A}$Rs. Specifically, $A_{2A}$Rs appear to exert both a facilitatory and a permissive role on mGlu5R-mediated effects.

The facilitatory role played by $A_{2A}$Rs towards mGlu5R-induced effects is demonstrated in hippocampal and corticostriatal slices. The synergistic reduction of the fEPSP slope in the hippocampus and of the FP amplitude in the striatum observed after the co-application of ineffective concentrations of CGS 21680 and CHPG was clearly superimposable, as well as the effect elicited by higher concentrations of CHPG (1 mM).

As for the mechanisms involved in the synergistic $A_{2A}$/mGlu5 receptor interaction, our results indicate the involvement of different transduction pathways in the striatum and hippocampus. Indeed, the potentiating effect of CGS 21680 was reproduced by forskolin and abolished by the PKA inhibitor KT 5720 in striatal but not in hippocampal slices, thus indicating that only in the former area does the $A_{2A}$/mGlu5 interaction require the recruitment of PKA. This finding is not so surprising since, although the cAMP/PKA pathway is considered the canonical transduction system operated by $A_{2A}$Rs [26], hippocampal...
A2A Rs also use a PKC-dependent transduction pathway, in particular in the control of glutamatergic transmission [23, 27]. On the other hand, the demonstration of an involvement of the cAMP/PKA pathway in the effects of striatal A2A Rs does not rule out other possible mechanisms, since A2A Rs can activate two signalling systems (one involving the activation of PKA and the other mediated by PKC), also in striatal nerve terminals [28]. However, we have not attempted to test whether PKC might be involved in this potentiation of mGlu5 responses by A2A Rs, since the manipulation of PKC activity is also expected to directly affect the responses mediated by mGlu5 Rs [29, 30].

In the striatum, both A2A and mGlu5 receptors are mainly localized postsynaptically [31, 32], although they have been found to colocalize in glutamatergic nerve terminals [8]. In the hippocampus, A2A and mGlu5 receptors are colocalized in glutamatergic synapses and they are present both in the presynaptic active zone and in the postsynaptic density, although with different subsynaptic distribution [19]. In spite of the ability of both A2A and mGlu5Rs to control the presynaptic release of glutamate [8, 21–23, 33–35], the findings obtained in both areas under a protocol of PPS (namely, the inability of CHPG and CGS 21680 to influence the R2/R1 ratio) suggest that the locus of their interaction is most probably postsynaptic. This possibility is reinforced by the predominant postsynaptic localization of mGlu5Rs, in particular in the hippocampus (e.g., [19, 36–38]), and by the finding that one of the main consequences of the interaction between A2A and mGlu5 receptors is the control of NMDA receptor-mediated effects (see below), which is likely to occur postsynaptically, because the postsynaptic density is the only neuronal compartment where the three receptors are simultaneously present [19].

In both hippocampal and striatal slices, we confirmed the previously reported potentiating effect of CHPG on NMDA-induced depolarization [39–41]. In both areas we demonstrated that the above effect was prevented not only by MPEP but also by the A2AR antagonist ZM 241385. Although the inhibitory effects of ZM 241385 were most prominent in the striatum, one of the most interesting observations was that the ability of CHPG to potentiate NMDA responses requires an endogenous tone at A2A receptors even in the hippocampus, i.e., a brain area in which the expression of such receptors is rather low. This was further strengthened by the finding that, in hippocampal slices from A2AR knockout (KO) mice, CHPG was unable to potentiate the fEPSP slope reduction induced by NMDA [19]. Furthermore, it is important to note that WT and A2AR KO mice showed similar responses to NMDA application, thus indicating that A2AR inactivation does not directly inhibit NMDA-mediated effects [19]. This is in agreement with the finding that, in rat slices, ZM 241385 prevented the potentiating effects of CHPG without directly inhibiting NMDA effects. Rather, in agreement with previous studies [25], we found that ZM 241385 tended to potentiate NMDA effects in corticostriatal slices.

The permissive role played by A2A Rs on mGlu5R-mediated effects appears to be also relevant in the modulation of NMDA-mediated toxicity. The findings obtained in cell cultures indicate that CHPG significantly potentiated NMDA-induced LDH release. This effect was blocked by MPEP, thus confirming the selective involvement of mGlu5Rs and, more interestingly, also by ZM 241385. This implies that the functional interaction between A2A and mGlu5Rs plays a significant role in the modulation of NMDA-mediated toxicity, an effect which could be particularly relevant in a region, such as the hippocampus, extremely sensitive to excitotoxic insults.

Altogether the present results suggest that the state of activation of A2A receptors may regulate the effects exerted by mGlu5Rs in terms of both synaptic plasticity [3, 15] and excitotoxicity [18, 42]. Another interesting implication is that the neuroprotective effects of A2AR antagonists towards neurodegeneration (see [43] for review) could be mediated not only by their modulation of glutamate outflow, but also by the removal of the permissive role of A2ARs on mGlu5R-mediated toxicity. In conclusion, the ability of A2ARs to control mGlu5R-dependent effects in different brain regions irrespective of their density emphasizes the role of A2ARs as a fine-tuning modulatory system [44] in the brain. These results add further support to the idea that A2ARs might represent an additional target for the development of therapeutic strategies against neurological disorders associated with NMDA receptor dysfunction.

References

1. Testa CM, Standaert DG, Landwehrmeyer GB et al (1995) Differential expression of mGluR5 metabotropic glutamate receptor mRNA by rat striatal neurons. J Comp Neurol 354:241–252
2. Popoli P, Pèzzola A, Torvinen M et al (2001) The selective mGlu5 receptor agonist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D2 receptors in the striatum: interaction with adenosine A2A receptors. Neuropsychopharmacology 25:505–513
3. Sung KW, Choi S, Lovinger DM (2001) Activation of group I mGluRs is necessary for induction of long-term depression at striatal synapses. J Neurophysiol 86:2405–2412
4. Bruno V, Ksiazek I, Battaglia G et al (2000) Selective blockade of metabotropic glutamate receptor subtype 5 is neuroprotective. Neuropharmacology 39(12):2223–2230
5. Cocchiello R, Brysse N, Almaric M (2004) Simultaneous blockade of adenosine A2A and metabotropic glutamate mGlu5 receptors increase their efficacy in reversing Parkinsonian deficits in rats. Neuropsychopharmacology 29:1451–1461
6. Kachroo A, Orlando LR, Grandy DK et al (2005) Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. J Neurosci 25(45):10414–10419
7. Díaz-Cabiale Z, Vivò M, Del Arco A et al (2002) Metabotropic
glutamate mGlu5 receptor-mediated modulation of the ventral striopallidal GABA pathway in rats: interactions with adenosine A2A and dopamine D2 receptors. Neurosci Lett 324:154–158

8. Rodrigues RJ, Alfaro TM, Rebola N et al (2005) Co-localization and functional interaction between adenosine A2A and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. J Neurochem 92:433–441

9. Ferré S, Karcz-Kubicha M, Hope B et al (2002) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proc Natl Acad Sci USA 99:11940–11945

10. Nishi A, Liu F, Matsuyama S et al (2003) Metabotropic mGlu5 receptors regulate adenosine A2A receptor signalling. Proc Natl Acad Sci USA 100:1322–1327

11. Cunha RA (2005) Neuroprotection by adenosine in the brain: from A1 receptor activation to A2A receptor blockade. Purinergic Signalling 1:111–134

12. Calabresi P, Centonze D, Pisani A, Bernardi G (1999) Metabotropic glutamate receptors and cell-type-specific vulnerability in the striatum: implications for ischemia and Huntington’s disease. Exp Neurol 158:97–108

13. Bruno V, Battaglia G, Copani A et al (2001) Metabotropic glutamate receptor subtypes as targets for neuroprotective drugs. J Cereb Blood Flow Metab 21(9):1013–1033

14. Gulberrini P, Sautelle E, Centonze D et al (2001) Selective involvement of mGlu1 in corticostriatal LTD. Neuropharmacology 40:1493–1503

15. Anwyrl R (1999) Metabotropic glutamate receptor localization: electrophysiological properties and role in plasticity. Brain Res Rev 29:83–120

16. Balschun D, Manahan-Vaughn D, Wagner T et al (1999) A specific role for group I mGluRs in hippocampal LTP and hippocampus-dependent spatial learning. Learn Mem 6:138–152

17. Sacaan AI, Scheppe DD (1992) Activation of hippocampal metabotropic excitatory amino acid receptors leads to seizures and neuronal damage. Neurosci Lett 139:77–82

18. Attucci S, Clodfelter GV, Thibault O et al (2002) Group I metabotropic glutamate receptor inhibition selectively blocks a prolonged Ca(2+)-elevation associated with age-dependent excitotoxicity. Neuroscience 112:183–194

19. Tebano MT, Martire A, Rebola N et al (2005) Adenosine A2A receptors and metabotropic glutamate 5 receptors are co-localized and functionally interact in the hippocampus: a possible key mechanism in the modulation of N-methyl-D-aspartate responses. J Neurochem 95:1188–1200

20. Domenici MR, Pepponi R, Martire A et al (2004) Permissive role of adenosine A2A receptors on metabotropic glutamate receptor 5 (mGluR5)-mediated effects in the striatum. J Neurochem 90:1276–1279

21. Popoli P, Betto P, Reggio R, Ricciarello G (1995) Adenosine A2A receptor stimulation enhances striatal extracellular glutamate levels in rats. Eur J Pharmacol 287:215–217

22. Pinto A, Pezzola A, Reggio R et al (2000) The mGlu5 receptor agonist CHPG stimulates striatal glutamate release: possible involvement of A2A receptors. Neuroreport 11:3611–3614

23. Lopes LV, Cunha RA, Kull B et al (2002) Adenosine A2A receptor facilitation of hippocampal synaptic transmission is dependent on tonic A1 receptor inhibition. Neuroscience 112:319–329

24. Schulz PE, Cook EP, Johnston D (1994) Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. J Neurosci 14:5325–5337

25. Tebano MT, Pinto A, Frank C et al (2004) Adenosine A2A receptor blockade differentially influences excitotoxic mechanisms at pre- and postsynaptic sites in the rat striatum. J Neurosci Res 77(1):100–107

26. Fredholm BB, Cunha RA, Svenssonsson P (2003) Pharmacology of adenosine A2A receptors and therapeutic applications. Curr Top Med Chem 3:413–426

27. Cunha RA, Ribeiro JA (2000) Adenosine A2A facilitation of synaptic transmission in the CA1 area of the rat hippocampus requires protein kinase C but not protein kinase A activation. Neurosci Lett 289:127–130

28. Gubitz AK, Widdowson L, Kurokawa M et al (1996) Dual signalling by the adenosine A2A receptor activation of both N- and P-type calcium channels by different G proteins and protein kinases in the same striatal nerve terminals. J Neurochem 67:374–381

29. Benquet P, Gee CE, Gerber U (2002) Two distinct signaling pathways upregulate NMDA receptor responses via two distinct metabotropic glutamate receptor subtypes. J Neurosci 22:9679–9686

30. Kotecha SA, Jackson MF, Al-Mahrouki A et al (2003) Co-stimulation of mGlu5R and N-methyl-D-aspartate receptors is required for potentiation of excitatory synaptic transmission in hippocampal neurons. J Biol Chem 278:27742–27749

31. Hettinger BD, Lee A, Linden J, Rosin DL (2001) Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. J Comp Neurol 431:331–346

32. Romano C, Sesma MA, McDonald CT et al (1995) Distribution of metabotropic glutamate receptor mGlu5 immunoreactivity in the rat brain. J Comp Neurol 355:455–469

33. Marchi M, Raiteri L, Risso F et al (2002) Effects of adenosine A1 and A2A receptor activation on the evoked release of glutamate from rat cerebrocortical synaphtosomes. Br J Pharmacol 136:434–440

34. Fazal A, Parker F, Palmer AM, Croucher MJ (2003) Characterisation of the actions of group I metabotropic glutamate receptor subtype selective ligands on excitatory amino acid release and sodium-dependent re-uptake in rat cerebrocortical synaphtosomes. J Neurochem 86:1346–1358

35. Wang SJ, Sihra TS (2004) Noncompetitive metabotropic glutamate5 receptor antagonist (E,2)-6-methyl-6-sulfo-2-pyridine (SIB1893) depresses glutamate release through inhibition of voltage-dependent Ca(2+) entry in rat cerebrocortical nerve terminals (synaphtosomes). J Pharmacol Exp Ther 309:951–958

36. Lujan R, Fusser Z, Roberts JD et al (1996) Perisynaptic localization of metabotropic glutamate receptors mGlu1R and mGlu5R on dendrites and dendritic spines in the rat hippocampus. Eur J Neurosci 8:1488–1500

37. Lujan R, Roberts JD, Shigemoto R et al (1997) Differential plasma membrane distribution of metabotropic glutamate receptors mGluR1 alpha, mGluR2 and mGluR5, relative to neurotransmitter release sites. J Chem Neuroanat 13:219–241

38. Shigemoto R, Kinosita A, Wada E et al (1997) Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J Neurosci 17:7503–7522

39. Dougherty AJ, Palmer MJ, Henley JM et al (1997) (R,S)-2-Chloro-5-hydroxyphenylglycine (CHPG) activates mGlu5, but not mGlu1, receptors expressed in CHO cells and potentiates NMDA responses in hippocampus. Neuropharmacology 36:265–267

40. Ugolini A, Corsi M, Bordi F (1999) Potentiation of NMDA and AMPA responses by the specific mGlu5R agonist CHPG in spinal cord motoneurons. Neuropharmacology 38:1569–1576

41. Pisani A, Gulberrini P, Bonsi P et al (2001) Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience 106:579–587

42. Orlando LR, Alsdorf SA, Penney JB, Young AB (2001) The role of group I and group II metabotropic glutamate receptors in modulation of striatal NMDA and quinolinic acid toxicity. Exp Neurol 167:196–204

43. Popoli P, Minghetti L, Tebano MT et al (2004) Adenosine A2A receptor antagonism and neuroprotection: mechanisms, lights, and shadows. Crit Rev Neurobiol 16(1–2):99–106

44. Sebastião AM, Ribeiro JA (2000) Fine-tuning neuromodulation by adenosine. Trends Pharmacol Sci 21:341–346