

# Is the functional interaction between adenosine A<sub>2A</sub> receptors and metabotropic glutamate 5 receptors a general mechanism in the brain? Differences and similarities between the striatum and the hippocampus

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**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<sub>2A</sub> receptors (A<sub>2A</sub>Rs) 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<sub>2A</sub>R 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. In both areas, a postsynaptic locus of interaction appeared more likely. *N*-methyl-D-aspartate (NMDA) reduced the fEPSP slope and FP amplitude in hippocampal and corticostriatal slices, respectively. Such an effect was significantly potentiated by CHPG in both areas. Interestingly, the A<sub>2A</sub>R 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<sub>2A</sub> 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<sub>2A</sub>Rs to control mGlu5R-dependent effects may thus be a general feature of A<sub>2A</sub>Rs 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<sub>2A</sub> receptors · extracellular recording · hippocampus · lactate dehydrogenase (LDH) · metabotropic glutamate 5 receptors (mGlu5Rs) · neuronal cultures · NMDA · protein kinase A · striatum

## Introduction

Adenosine A<sub>2A</sub> receptors (A<sub>2A</sub>Rs) 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<sub>2A</sub>Rs have been reported in

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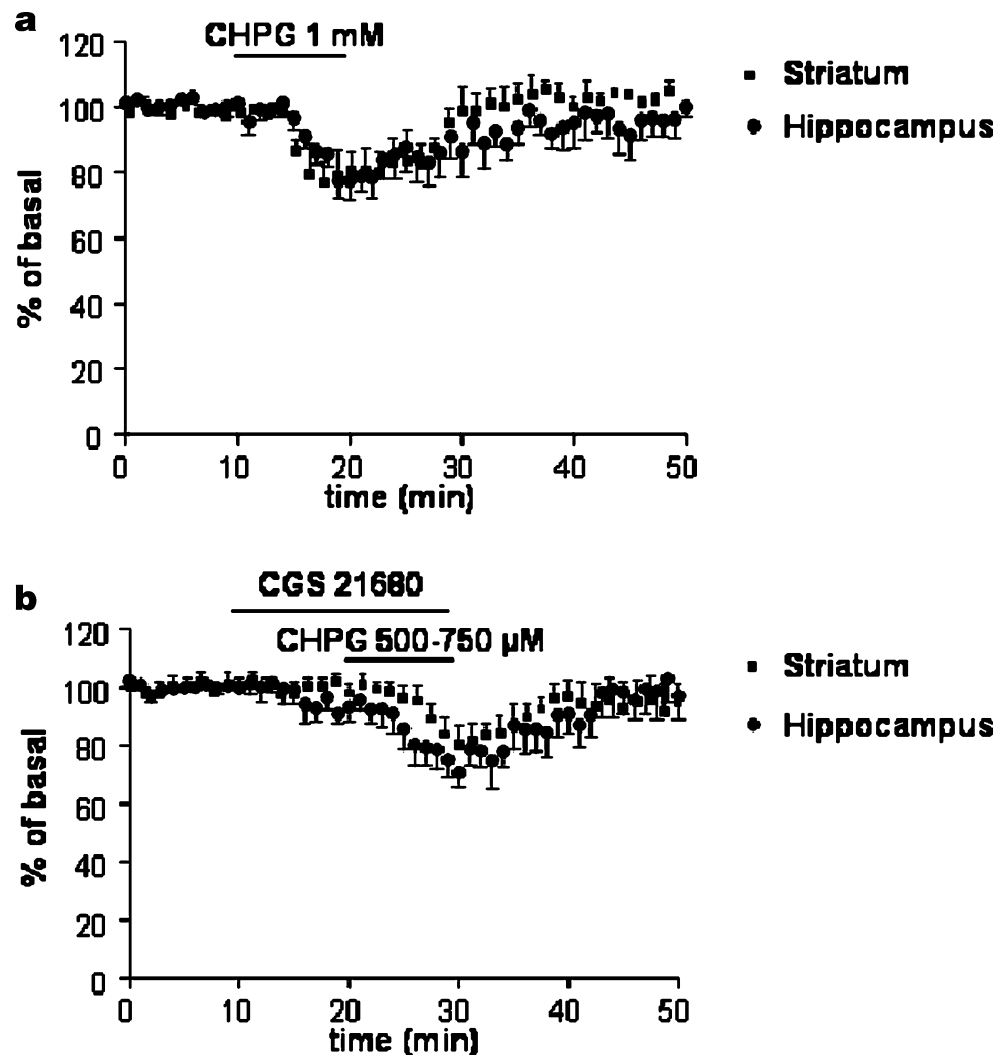
the modulation of several effects, such as motor behavior in models of Parkinson's disease [2, 5, 6],  $\gamma$ -aminobutyric acid (GABA) and glutamate release [7, 8], *c-fos* expression [9], and DARPP-32 phosphorylation [10]. Such functional interactions were supported by the demonstration of the existence of heteromeric complexes containing  $A_{2A}$  and mGlu5 receptors in striatal neurons [9].

Although adenosine  $A_{2A}$  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  $A_{2A}$  and mGlu5Rs are colocalized in the rat hippocampus [19].

The activation of  $A_{2A}$  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  $\mu$ M concentrations. As shown in Figure 1a, only at concentrations of 1 mM did CHPG induce a reduction of the fEPSPs slope in the hippocampus ( $-21.8 \pm 4\%$  of basal) and of the FP amplitude in the striatum ( $-15.7 \pm 5.4\%$ ); in both cases, CHPG-induced reductions completely

**Figure 1** Adenosine  $A_{2A}$  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  $\mu$ M), 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  $\pm$  SEM). The period of drug application is indicated by the horizontal bars



recovered after washout. The effect of CHPG was significantly reduced by the selective mGlu5R antagonist MPEP (30–50  $\mu\text{M}$ ) in both the hippocampus and the striatum (MPEP + CHPG:  $-7.1 \pm 1.5\%$  of basal,  $P < 0.01$  vs CHPG alone in the hippocampus; MPEP + CHPG:  $-3.53 \pm 0.44\%$  of basal,  $P < 0.05$  vs CHPG alone in the striatum).

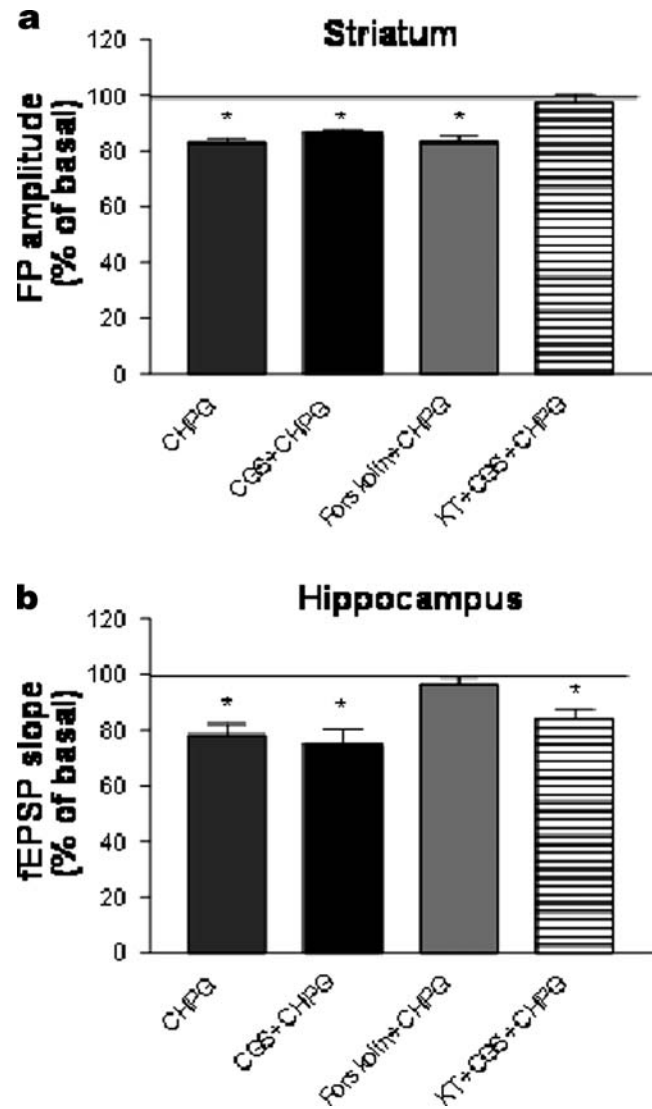
To investigate whether the activation of  $A_{2A}$ Rs could have a facilitatory role on mGlu5R-mediated effects, CGS 21680, a selective  $A_{2A}$ R agonist, was used. Combined ineffective doses of CHPG and CGS 21680 (500  $\mu\text{M}$  + 50 nM in the hippocampus; 750  $\mu\text{M}$  + 30 nM in the striatum) synergistically reduced the slope of fEPSPs recorded in CA1 ( $-25.0 \pm 5.4\%$ ,  $P < 0.005$  vs basal) and the amplitude of field potentials (FPs) recorded in the dorsomedial striatum ( $-13.42 \pm 2.7\%$ ,  $P < 0.05$  vs basal), whereas neither CGS 21680 nor CHPG affected synaptic transmission on their own (Figure 1b). Both MPEP (30  $\mu\text{M}$ ) and the selective  $A_{2A}$ R antagonist ZM 241385 (100 nM) abolished the synergistic effect resulting from the coactivation of  $A_{2A}$  and mGlu5Rs (not shown).

Different mechanisms are involved in the  $A_{2A}$ /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 ( $-17.99 \pm 4.89\%$  with respect to basal) in corticostriatal slices (Figure 2a,b). Conversely, forskolin (up to 30  $\mu\text{M}$ ) 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  $\mu\text{M}$ ) only in the striatum. These results indicate an involvement of the cyclic adenosine monophosphate (cAMP)/PKA pathway in the  $A_{2A}$ /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  $A_{2A}$  and mGlu5Rs modulate glutamate release [21–23], we wanted to establish the possible involvement of presynaptic mechanisms in the effects of  $A_{2A}$  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,



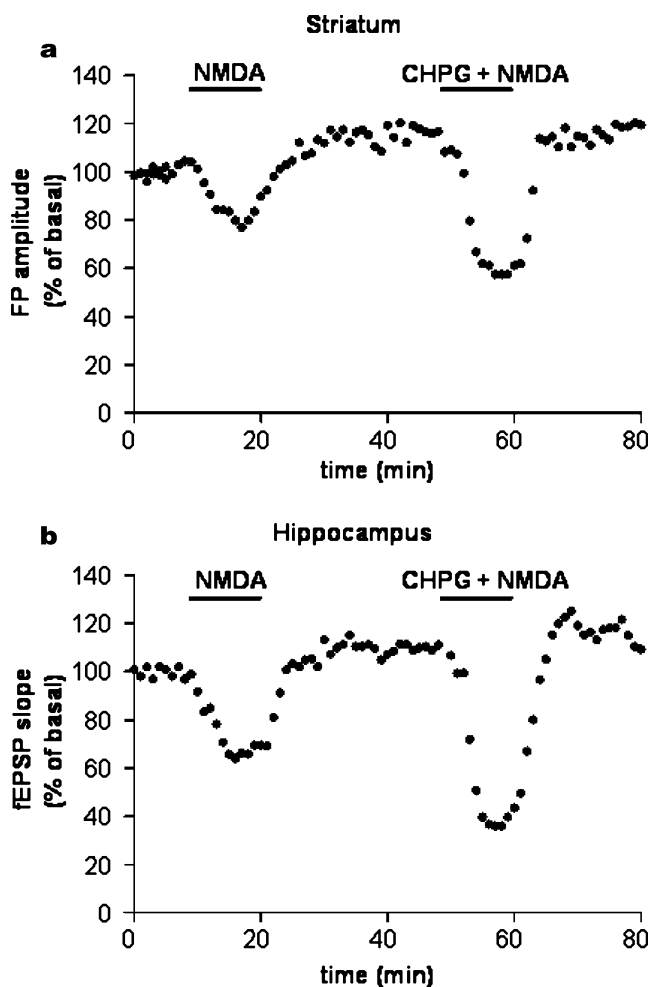
**Figure 2** A different mechanism is involved in the synergism between  $A_{2A}$  and mGlu5 receptors in hippocampal and corticostriatal slices. The histograms show that the effect of CGS 21680 is mimicked by the adenylate cyclase activator forskolin (100 nM) and blocked by the PKA inhibitor KT 5720 (4  $\mu\text{M}$ ) in the striatum (a) but not in the hippocampus (b). The results are expressed as means  $\pm$  SEM of 4–6 experiments. \* $P < 0.05$  vs baseline (Wilcoxon signed rank test)

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

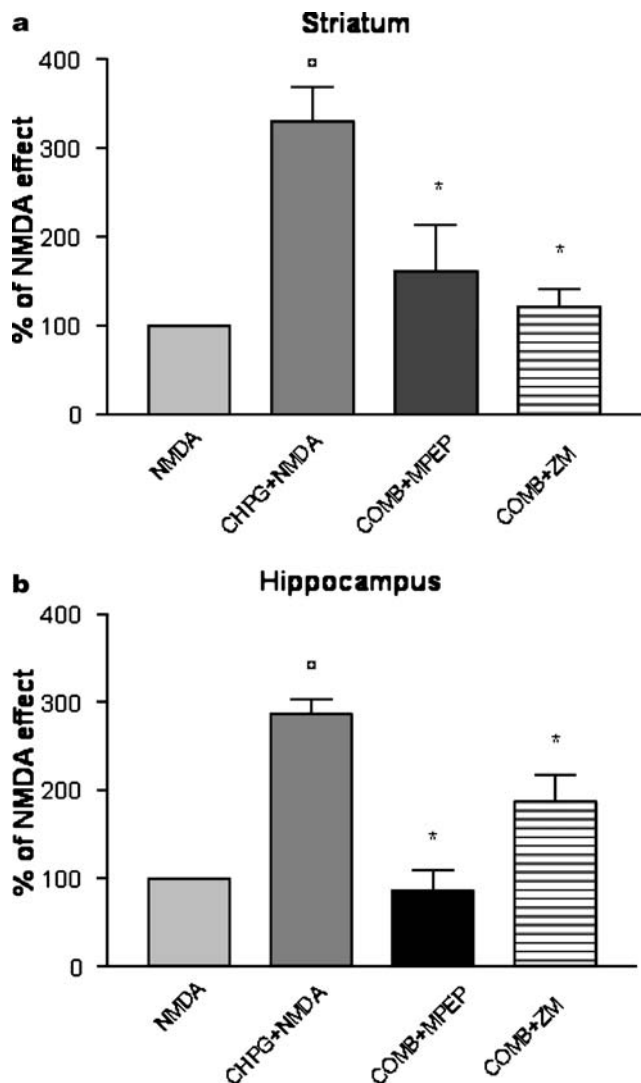
R2/R1 ratio during the PPS protocol ( $P > 0.05$  vs control in both areas, data not shown). Thus, although a lack of effect upon paired pulse stimulation does not allow to definitely exclude a presynaptic locus of action, such a possibility appears not very likely.

In both brain areas, the state of activation of  $A_{2A}$  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 ( $\approx 27\%$ ) and of the FP amplitude ( $\approx 23\%$ ) in hippocampal and corticostriatal slices, respectively. Such an effect was strongly potentiated by the application of CHPG 500–750  $\mu\text{M}$

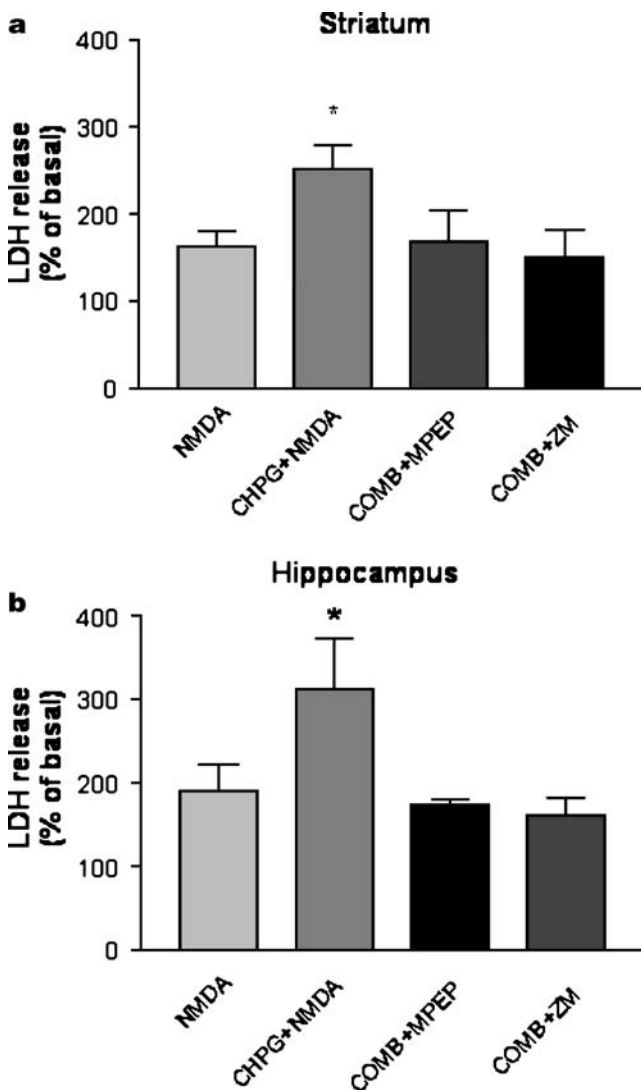


**Figure 3** CHPG-potentiated NMDA effects in hippocampal and corticostriatal slices. Graphs represent the time course of changes in FP amplitude and fEPSP slope in two typical experiments recorded in corticostriatal (a) and in hippocampal slices (b), respectively. A 10-min superfusion period with NMDA (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



**Figure 4** The blockade of either  $A_{2A}$  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  $A_{2A}$  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.  $^{\circ}P < 0.05$  (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  $A_{2A}$ R 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



**Figure 5** The blockade of either  $A_{2A}$  or mGlu5 receptors prevents the ability of CHPG to potentiate NMDA-induced LDH release from cultured hippocampal and striatal neurons. The application of NMDA (300  $\mu$ M for 60 min) to both striatal and hippocampal neurons induced a significant increase in LDH release. The mGlu5R agonist CHPG (1 mM) was devoid of effects on its own, but it significantly potentiated NMDA-induced LDH release. The mGlu5R antagonist MPEP (30  $\mu$ M) or the  $A_{2A}$  receptor antagonist ZM 241385 (30 nM) were devoid of effects by themselves but prevented the ability of CHPG to potentiate the release of LDH. Results represent means  $\pm$  SEM values of 3–4 independent experiments, assayed in triplicate. \* $P < 0.05$  (Mann-Whitney U test) vs NMDA alone

241385 (100 nM) fully prevented CHPG-induced potentiation of NMDA effects ( $P < 0.05$  vs NMDA + CHPG, Figure 4b). Interestingly, ZM 241385 alone did not influence basal synaptic transmission nor did it directly inhibit NMDA effects but instead, in agreement with previous observations from our group [25], it tended to increase NMDA-induced FP depression ( $165.91 \pm 31.03\%$ ;  $P < 0.05$  vs NMDA alone).

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  $\mu$ M NMDA induced a comparable increase of LDH release in hippocampal ( $190.2 \pm 32.4\%$ ) and striatal cell cultures ( $170.3 \pm 18.46$ ). CHPG 1 mM (ineffective by itself) significantly potentiated NMDA-induced LDH release ( $312.4 \pm 60.1\%$  in the hippocampus;  $249.5 \pm 31.64\%$  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  $\mu$ M) or ZM 241385 (30 nM) were able to restore the same levels of LDH induced by NMDA alone ( $P < 0.05$  vs CHPG  $\pm$  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



A<sub>2A</sub>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 A<sub>2A</sub>Rs does not rule out other possible mechanisms, since A<sub>2A</sub>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 A<sub>2A</sub>Rs, since the manipulation of PKC activity is also expected to directly affect the responses mediated by mGlu5Rs [29, 30].

In the striatum, both A<sub>2A</sub> and mGlu5 receptors are mainly localized postsynaptically [31, 32], although they have been found to colocalize in glutamatergic nerve terminals [8]. In the hippocampus, A<sub>2A</sub> 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 A<sub>2A</sub> 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 A<sub>2A</sub> 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 A<sub>2A</sub>R 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 A<sub>2A</sub> 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 A<sub>2A</sub>R 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 A<sub>2A</sub>R KO mice showed similar responses to NMDA application, thus indicating that A<sub>2A</sub>R 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 A<sub>2A</sub>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 A<sub>2A</sub> 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 A<sub>2A</sub> 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 A<sub>2A</sub>R 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 A<sub>2A</sub>Rs on mGlu5R-mediated toxicity. In conclusion, the ability of A<sub>2A</sub>Rs to control mGlu5R-dependent effects in different brain regions irrespective of their density emphasizes the role of A<sub>2A</sub>Rs as a fine-tuning modulatory system [44] in the brain. These results add further support to the idea that A<sub>2A</sub>Rs might represent an additional target for the development of therapeutic strategies against neurological disorders associated with NMDA receptor dysfunction.

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