British Journal of Pharmacology (2003) 138, 1059–1068
www.nature.com/bjp

Interactions between adenosine and metabotropic glutamate receptors in the rat hippocampal slice

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1 We have examined excitatory postsynaptic potentials and paired-pulse interactions in rat hippocampal slices to obtain more information about the site and mechanism of interactions between metabotropic glutamate receptors and adenosine receptors.

2 The results show that the suppression of adenosine sensitivity is explained by a selectively reduced responsiveness to A_1 receptor stimulation, and does not involve any facilitation of A_{2A} adenosine receptors, since it can be obtained in the absence of endogenous adenosine and is not prevented by the A_{2A} receptor blocker ZM241385.

3 The glutamate receptors involved are of the group I class since the suppression of adenosine sensitivity is produced by ACPD and the group I selective compound DHPG. Furthermore, the effects of DHPG could be prevented by LY367385, a selective antagonist at the $mGlu_{1a}$ subtype of group I receptors. The selective antagonist at $mGlu_5$ receptors, SIB1893, did not prevent the suppression of adenosine sensitivity by DHPG. Blockade of the DHPG/adenosine interaction was also obtained by superfusion with the protein kinasae C inhibitor chelerythrine.

4 Since the suppression of adenosine responses by metabotropic receptor agonists was seen in the paired-pulse paradigm, we conclude that the observed interactions occur at the level of the presynaptic terminals.

5 The interaction with adenosine receptors is not specific, but applies also to a suppression of responses mediated by the $GABA_B$ receptor agonist baclofen.

6 We conclude that activation of the mGlu1a subtype of receptor can suppress responses mediated via adenosine A1 receptors, probably by activating protein kinase C. Since the changes induced by metabotropic glutamate receptor agonists last for at least 60 min, the data also imply that these interactions could play an important role in changes of synaptic function long after even transient increases of glutamate release in the CNS.
British Journal of Pharmacology (2003) **138,** 1059–1068. doi:10.1038/sj.bjp.0705083

Keywords: Adenosine; metabotropic receptors; glutamate; protein kinase C; chelerythrine; paired-pulse

Abbreviations: 2AP5, D-2-amino-5-phosphono-pentanoic acid; ACPD, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; ACSF, artificial cerebrospinal fluid; CPA, 8-cyclopentyladenosine; DHPG, (*RS*)-3,5-dihydroxyphenylglycine; EPSPs, excitatory postsynaptic potentials; GF109203X, 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl)maleimide; KT5720, (9*R*,10*S*,12*S*)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1*H*-di-indolo[1,2,3-*fg*:3′,2′,1′-*kl*]pyrrolo[3,4*l*][1,6]benzodiazocine-10-carboxylic acid hexyl ester; LTD, long-term depression; LTP, long-term potentiation; LY367385, (*S*)-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid; NMDA, *N*-methyl-D-aspartate; SC-9, 5-chloro-*N*-(6-phenylhexyl)-1-naphthalenesulphonamide; SIB1893, 2-methyl-6-(2-phenylethenyl)pyridine; ZM241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3*a*][1,3,5]triazin-5-ylamino]ethyl)phenol

Introduction

Metabotropic glutamate receptors (mGluRs) are a family of excitatory amino-acid receptors linked to G-protein-coupled transduction systems. At least eight mGluR subtypes have been cloned and they can be classified into three groups based on amino acid homology, transducing mechanisms and the pharmacology of the receptors (Nakanishi, 1994; Conn & Pin, 1997). The mGluRs play an important role in the regulation of synaptic transmission via their modulation of ion channels and ionotropic glutamate receptors in diverse neuronal cell types (Pin & Duvoisin, 1995; Conn & Pin, 1997). Activation of mGluRs decreases current through voltage-dependent calcium channels in rat hippocampal (Swartz & Bean, 1992) and striatal neurons (Stefani *et al*., 1994). Different subtypes of potassium channels may also be modulated by mGluRs in several types of neuronal cells (Conn & Pin, 1997).

The mGluRs also have a role in various forms of synaptic plasticity. It has been reported that the induction of long-term potentiation (LTP) in the hippocampus requires synaptic activation of mGluRs (Bashir *et al*., 1993; Riedel *et al*., 1995), and hippocampal LTP is greatly reduced in mGluR1 knockout mice (Aiba *et al*., 1994). MGluRs are also involved in the induction of long-term depression (LTD) at parallel fiber–Purkinje cell synapses in the cerebellum (Linden & Connor, 1993; Hartell, 1994). Activation of group I mGluRs with the selective agonist (*RS*)-3,5-dihydroxyphenylglycine

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(DHPG) induces LTD in the CA1 region of hippocampal slices in Mg^{2+}-free medium or during blockade of GABA-mediated inhibition.

There are several experimental and pathological situations, such as hypoxia or ischaemia, in which there is increased release of glutamate from cells. In many of these similar circumstances, there is also an increase in the extracellular levels of adenosine (Stone & Simmonds, 1991; Latini & Pedata, 2001). Interactions between adenosine receptors and ionotropic excitatory amino-acid transmission (via *N*-methyl-D-aspartate (NMDA) receptors) have previously been reported from our laboratory (Bartrup & Stone, 1988, 1990; Nikbakht & Stone, 2001), and there have been reports of interactions between adenosine and metabotropic glutamate receptors (de Mendonca & Ribeiro, 1997; Budd & Nicholls, 1995). It is not clear, however, whether the reported interactions are specific for adenosine receptors, and whether they occur primarily at presynaptic or postsynaptic sites. We have now, therefore, extended the earlier work by studying paired-pulse interactions, comparing the effects of adenosine and the $GABA_B$ receptors agonist baclofen, identifying the nature of the adenosine and glutamate receptors involved, and examining the time course of the interactions.

Methods

Hippocampal slices were prepared from male Wistar rats (150–200 g). Animals were anaesthetized with urethane (1.3 g kg^{-1}, i.p.), killed by cervical dislocation, decapitated and the brains removed into chilled artificial cerebrospinal fluid (ACSF) gassed with 95% O_2 and 5% CO_2 and containing (in mM) KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10. The hippocampi were dissected free of surrounding tissue and were cut transversely into slices 450 μm thick using a McIIwain tissue chopper. Slices were maintained in an incubation chamber at room temperature in an atmosphere of 95% O_2 and 5% CO_2 for at least 1 h prior to recording.

For recording, individual slices were transferred to the recording chamber where they were continuously superfused while submerged at a rate of 3 ml min^{-1} with the same gassed solution at 30°C. Drugs were added to this superfusing solution. Extracellular population excitatory postsynaptic potentials (EPSPs) were recorded in the CA1 pyramidal cell layer using single glass microelectrodes filled with 2 M NaCl (tip diameter approximately 2–4 μm, DC resistance <5 MΩ). The tip of a concentric bipolar stimulating electrode was positioned in the stratum radiatum for orthodromic activation of pyramidal cells. Stimuli were square-wave constant-current pulses of 300 μs duration. Paired stimuli were delivered through the same electrode. Evoked responses were amplified, displayed on digital storage oscilloscopes and plotted onto a computer by use of a Cambridge Electronic Design (CED) interface and analysis software.

The EPSPs were quantified in terms of the slope of the initial phase of the potential. When paired-pulse inhibition was examined, inhibition is expressed as the change in the second response of a pair compared with the first response. The significance of the difference between two means was calculated by use of a *t*-test, paired or nonpaired as appropriate. The significance of the differences between more than two means obtained in the same slices was calculated with repeated measures ANOVA followed by the Student–Newman–Keuls test. *P* values less than 0.05 were considered statistically significant. Drugs were obtained from Tocris Chemicals Ltd.

Results

From previous work, a concentration of 10 μM adenosine was chosen for most of these experiments, as it produced an approximately 50% inhibition of EPSP slope, from which increases or decreases could readily be observed and quantified. Adenosine 10 mM depressed single EPSPs by $59.35 \pm 3.39\%$ ($P<0.001$; $n=4$; ANOVA followed by Student–Newman–Keuls test) relative to the initial control size (Figure 1). When applied alone, the nonselective mGluR agonist (1*S*, 3*R*)-1-aminocyclopentare-1,3,-dicarboxylic acid (ACPD) (100 μM) reduced the EPSP size by 80.83% (Figure 1a).

When a series of adenosine responses was obtained, and an application of ACPD made after the first of these, it was found that the subsequent responses were reduced significantly in size. EPSP slope was depressed by only 38.7% by an application of adenosine 20 min after the ACPD perfusion ($P<0.01$ compared with the initial adenosine response, $n=4$) (Figure 1a). Adenosine responses obtained 40 and 60 min after the ACPD application remained significantly smaller than the initial response ($P<0.05$) (Figure 1a). The normal reproducibility of a series of adenosine or baclofen applications is illustrated by the three control responses obtained before the superfusion with ACPD (Figure 1a,c).

Paired-pulse interactions

In the paired-pulse paradigm, interstimulus intervals of 10, 20 and 50 ms were tested that have previously been shown to generate paired-pulse inhibition (at 10 ms) and later paired-pulse potentiation (20 and 50 ms) (Higgins & Stone, 1996; Nikbakht & Stone, 2001). Superfusion of adenosine alone converted the inhibition to facilitation at 10 ms and produced a marked increase in facilitation at 20 and 50 ms (Figures 1b and 2). All these changes were highly significant ($P<0.001$; $n=4$; ANOVA followed by Student–Newman–Keuls test). Applied alone, ACPD tended to produce a similar shift but this did not reach statistical significance. When adenosine was tested 20 min following a 10 min application of ACPD, its effects were again reduced significantly (Figure 1b; $P<0.05$ compared with the initial adenosine response; $n=4$). Subsequent responses to adenosine gradually returned towards the original size over a 60 min period (data not shown for clarity), as in the study of EPSP slope size described above.

Responses to baclofen

In order to determine whether this lasting reduction of sensitivity was specific for adenosine, similar experiments were performed using ACPD 100 μM and baclofen 2 μM. Baclofen initially depressed the single EPSP slope by 59.86% of the basal potential (Figure 1c) whereas, after perfusing ACPD, baclofen reduced the EPSP slope by only 41.66, 43.61 and 47.57% after 20, 40 and 60 min, respectively. ACPD also

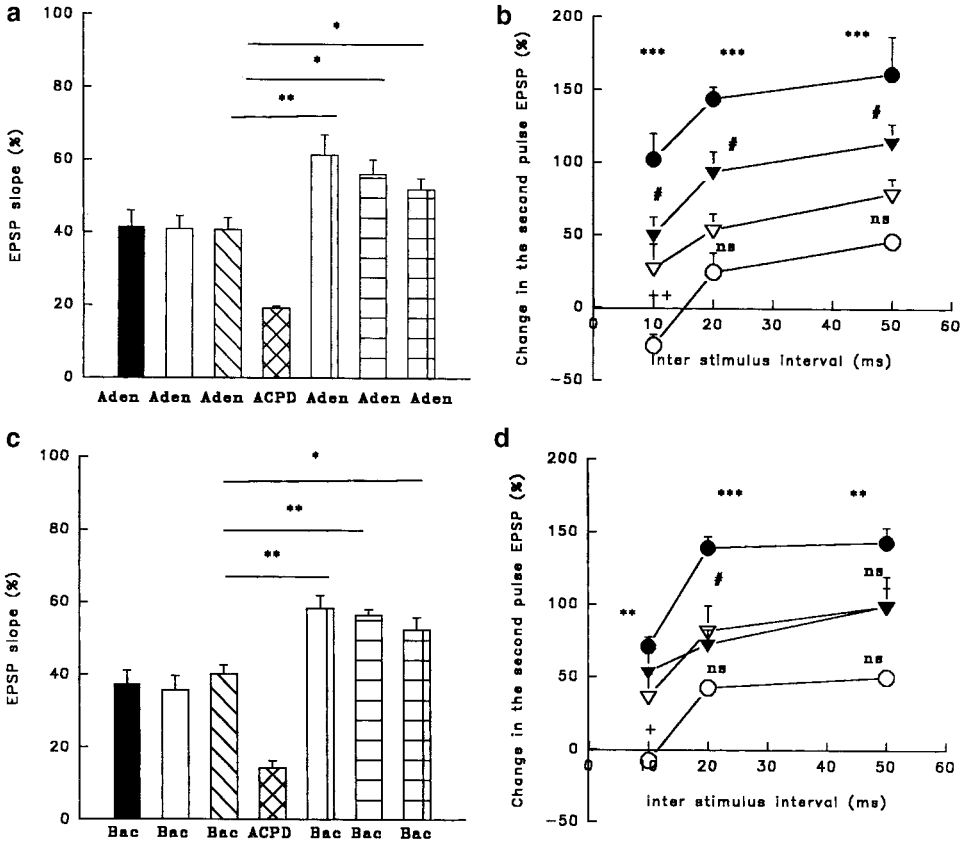

Figure 1 (a) Histogram showing the interaction between adenosine (10 μM) and ACPD (100 μM) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of a series of three control applications of adenosine alone (Aden), the effect of ACPD alone, and the reduced responses to adenosine observed 20, 40 and 60 min after the application of ACPD. The columns indicate the mean$\pm$s.e.m. ($n=4$). Statistically significant differences between columns are indicated as $^{*}P<0.05$, $^{**}P<0.01$ (ANOVA followed by Student–Newman–Keuls test). (b) Graph showing the changes of paired-pulse response size (the % change in the second of a pair of responses relative to the first). Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of adenosine 10 μM, ACPD 100 μM and adenosine 10 μM after ACPD. Only the first adenosine response after ACPD, obtained at 20 min, is shown for clarity. Each point shows the mean$\pm$s.e.m. for $n=4$ slices. $^{***}P<0.001$ between controls and adenosine before ACPD; $\#P<0.05$ between adenosine responses before and after ACPD (ANOVA followed by Student–Newman–Keuls test). (c) Histogram showing the interaction between baclofen (2 μM) and ACPD (100 μM) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of a series of three control applications of baclofen alone (Bac), the effect of ACPD alone, and the reduced responses to baclofen observed 20, 40 and 60 min after the application of ACPD. The columns indicate the mean$\pm$s.e.m. ($n=4$). Statistically significant differences between columns are indicated as $^{*}P<0.05$, $^{**}P<0.01$. (d) Graph showing the changes of paired-pulse response size (the % change in the second of a pair of responses relative to the first). Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of baclofen 2 μM, ACPD 100 μM and baclofen 2 μM following ACPD. Only the first baclofen response after ACPD, obtained at 20 min, is shown for clarity. Each point shows the mean$\pm$s.e.m. for $n=4$ slices. $^{***}P<0.001$ between controls and baclofen responses before ACPD; $\#P<0.05$ between baclofen responses before and after ACPD (ANOVA followed by Student–Newman–Keuls test).

reduced significantly the decrease in paired-pulse inhibition and later paired-pulse facilitation produced by baclofen (Figure 1d).

Receptor identification

In order to determine whether group I mGluRs were responsible for the effect of ACPD, the selective agonist DHPG was used. Before DHPG, adenosine 10 μM depressed EPSP size by 58.74$\pm$3.32% of the basal level ($P<0.01$; $n=5$) in this series of experiments. DHPG (10 μM) alone decreased the EPSP slope by 40.18$\pm$4.29% ($P<0.001$; $n=5$). After perfusing DHPG 10 μM, the responses to adenosine were reduced significantly after 20 and 40 min, respectively. The time course of these changes is illustrated in Figure 3a, together with sample records of the population EPSP slope (Figure 3b).

Adenosine at 10 μM removed any early paired-pulse inhibition and enhanced later facilitation ($P<0.001$; $n=5$). DHPG had no significant effect alone, but it reduced the effect of adenosine at both the 10 and 20 ms stimulus intervals. As in the earlier studies above, sensitivity to adenosine gradually recovered so that, after 60 min, responses were not different from the original size (data not shown for clarity).

Baclofen (2 μM) depressed the single EPSP slope by 69.11$\pm$3.37% of the basal potential ($P<0.001$; $n=4$) whereas, at 20 and 40 min after perfusion with DHPG, it reduced EPSP slope significantly less. In the paired-pulse experiments, DHPG tended to reduce the effect of baclofen, although this did not reach significance.

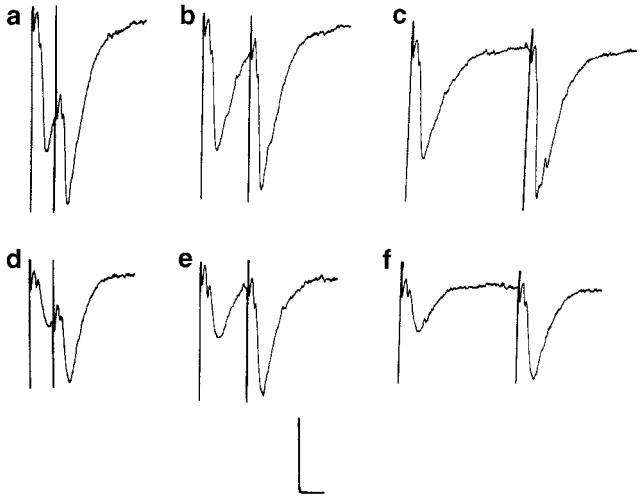

Figure 2 Records of the population EPSP during the induction of paired-pulse interactions, showing control responses at interstimulus intervals of (a) 10 ms, (b) 20 ms and (c) 50 ms. The lower traces (d–f) illustrate the effect of adenosine, 10 μM at the same stimulus intervals. Calibrations: 1 mV and 10 ms.

Two selective antagonists were used to assess the involvement of subtypes of the group I mGluRs. (*S*)-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid (LY367385), an antagonist at $mGlu_{1a}$ receptors, was superfused at 100 μM. This compound had no effect itself on the baseline size of the EPSP slope, and did not modify sensitivity to adenosine. It did, however, prevent the direct inhibitory activity of DHPG itself on the EPSP slope (Figure 4a) and on paired-pulse interactions (Figure 4b). It also prevented completely the depression by DHPG of adenosine sensitivity, so that the effect of adenosine on EPSP slope and paired-pulse interaction was not different from that recorded before the superfusion of DHPG (Figure 4a,b).

In contrast, the $mGlu_5$ antagonist 2-methyl-6-(2-phenylethenyl)pyridine (SIB1893) at 40 μM did not prevent the reduction of adenosine sensitivity by DHPG, either on EPSP slope or paired-pulse interactions (Figure 4c,d).

Involvement of protein kinases

The involvement of protein kinase C (PKC) in the effects of the mGluR ligands was examined using the PKC inhibitors 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl)malcimide (GF109203X) and chelerythrine. Both were applied for 10 min before, during and following the application of DHPG. At a concentration of 100 nM, GF109203X had no effect alone or on the responses to adenosine, but it did prevent the depression of adenosine responses caused by DHPG (Figure 5a). At 5 μM, chelerythrine similarly had no significant effect of its own but did prevent the suppression of adenosine responses by DHPG on the single EPSP slope (Figure 5c). In addition, GF109203X and chelerythrine prevented the changes by DHPG of adenosine effects on paired-pulse interactions (Figure 5b,d), such that these remained at the initial control size for up to 60 min after the application of DHPG. In contrast, the protein kinase A inhibitor (9*R*, 10*S*, 12*S*)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1*H*-di-indolo[1,2,3-*fg*:3′,2′,1′-*kl*]pyrrolo[3,4*l*][1,6]benzodiazocine-10-carboxylic acid hexyl ester (KT5720) did not interfere with the interaction between DHPG and adenosine (Figure 6a,b).

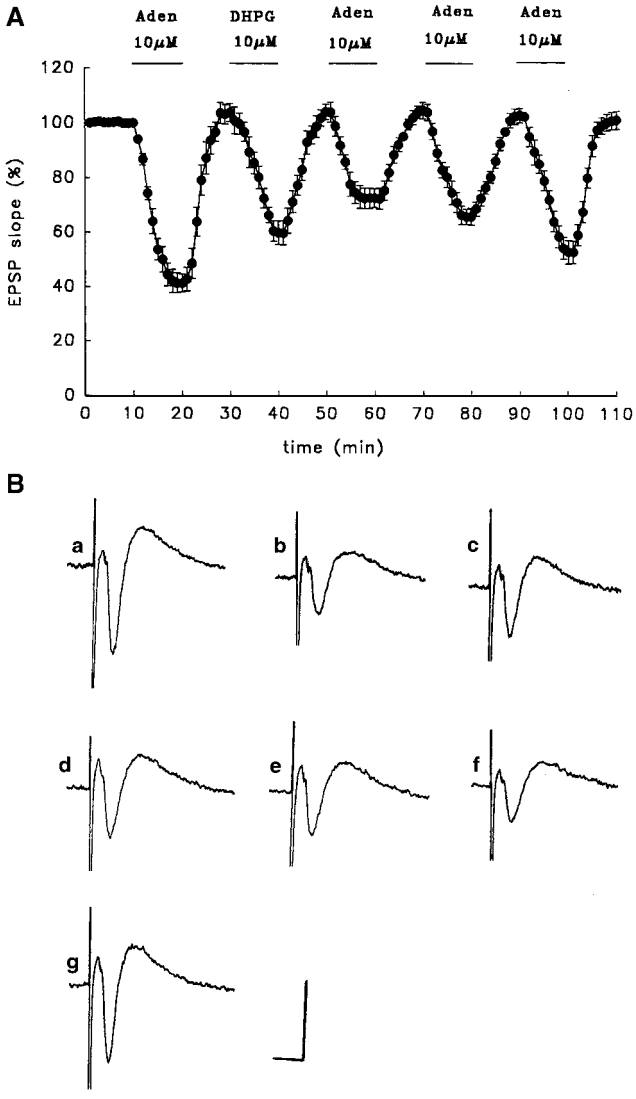

Figure 3 (A) Time course of recovery of the EPSP slope after adenosine and DHPG applications, and of the depression of adenosine responses following DHPG. Each point is the mean ± s.e.m. of four experiments. (B) Representative original recordings taken (a) at the start and (g) end of the sequence illustrated in (A), with intervening records taken at the peaks of the adenosine and DHPG responses, respectively. Calibrations: 1 mV and 10 ms.

As a further test of the possible role of PKC, the PKC activator SC-9 (10 μM) was superfused for 10 min before, during and after a test application of adenosine. This resulted in a significant reduction in the size of the response to adenosine, examined on a single EPSP slope (Figure 6c) or on paired-pulse interactions (Figure 6d).

Identification of adenosine receptors

In order to determine whether the effect of mGluR activation on adenosine responses was mediated via an action specifically on A_1 adenosine receptors, experiments were performed in

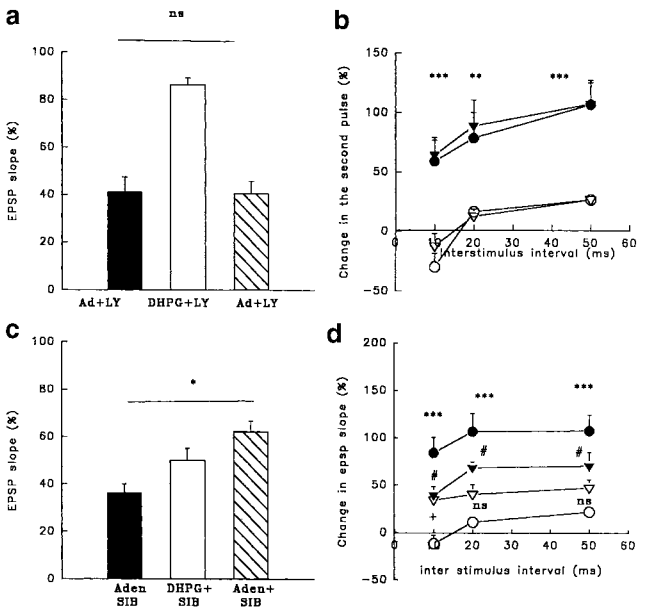

Figure 4 (a) Histogram showing the interaction between adenosine (10 μM) and DHPG (10 μM) in the presence of LY367385 (100 μM) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of adenosine in the presence of LY367385 (Ad + LY), the effect of DHPG in the presence of LY367385 and the normal response to adenosine observed 20 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n = 4$). (b) Graph showing the changes of paired-pulse response. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of adenosine 10 μM in the presence of LY367385, DHPG 10 μM in the presence of LY367385 and adenosine 10 μM following DHPG obtained at 20 min. Each point shows the mean ± s.e.m. for $n = 4$ slices. $^{***}P < 0.001$ and $^{**}P < 0.01$ between controls and adenosine in the presence of LY367385 before DHPG (ANOVA followed by Student–Newman–Keuls test). (c) Histogram showing the interaction between adenosine (10 μM) and DHPG (10 μM) on the EPSP slope in hippocampal slices in the presence of SIB1893 (40 μM). The columns summarise, respectively, the inhibitory effect of adenosine in the presence of SIB1893, the effect of DHPG in the presence of SIB1893 and the reduced response to adenosine observed 20 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n = 4$). Statistically significant differences between columns are indicated as $^{*}P < 0.05$ (ANOVA followed by Student–Newman–Keuls test). (d) Graph showing the changes of paired-pulse response size. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of adenosine 10 μM in the presence of SIB1893, DHPG 10 μM in the presence of SIB1893 and adenosine 10 μM following DHPG. Each point shows the mean ± s.e.m. for $n = 4$ slices. $^{***}P < 0.001$ between controls and adenosine in the presence of SIB1893; $\#P < 0.05$ between adenosine before and after DHPG in the presence of SIB1893 (ANOVA followed by Student–Newman–Keuls test).

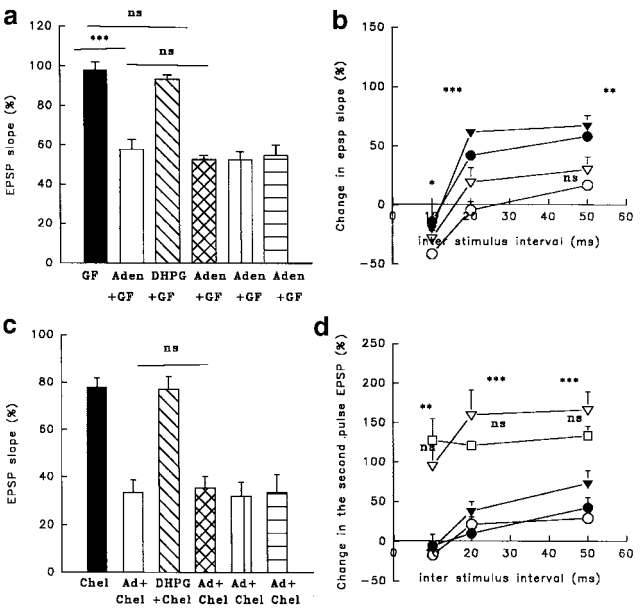

Figure 5 (a) Histogram showing the influence of GF109203X (100 nM) on the interaction between adenosine (10 μM) and DHPG (10 μM) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of GF109203X 100 nM alone (GF), the effect of adenosine in the presence of GF109203X, DHPG in the presence of GF109203X, and the normal responses to adenosine observed 20, 40 and 60 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n = 4$). (b) Graph showing the changes of paired-pulse response. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, adenosine in the presence of GF109203X, DHPG in the presence of GF109203X and adenosine 10 μM following DHPG. Only the first adenosine response after DHPG, obtained at 20 min, is shown for clarity. Each point shows the mean ± s.e.m. for $n = 4$ slices. $^{***}P < 0.001$ and $^{**}P < 0.01$ between controls and adenosine before DHPG (ANOVA followed by Student–Newman–Keuls test). (c) Histogram showing the influence of chelerythrine 5 μM (Chel) on the interaction between adenosine 10 μM and DHPG 10 μM on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of chelerythrine alone (Chel), the effect of adenosine in the presence of chelerythrine, DHPG in the presence of chelerythrine, and the normal responses to adenosine observed 20, 40 and 60 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n = 4$). (d) Graph showing the changes of paired-pulse response size. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of chelerythrine alone, adenosine in the presence of chelerythrine, DHPG in the presence of chelerythrine and adenosine 10 μM following DHPG. Only the first adenosine response after DHPG, obtained at 20 min, is shown for clarity. Each point shows the mean ± s.e.m. for $n = 4$ slices. $^{***}P < 0.001$ and $^{**}P < 0.01$ between controls and adenosine before DHPG (ANOVA followed by Student–Newman–Keuls test).

which slices were superfused with adenosine deaminase (0.1 U ml^{-1}) to remove endogenous adenosine, and A_1 receptors were then activated using the selective agonist 8-cyclopentyladenosine (CPA). When superfused alone, CPA 20 nM significantly depressed the EPSP slope (Figure 7a) and enhanced paired-pulse facilitation (Figure 7b) as has been reported in previous studies (Nikbakht & Stone, 2001). Adenosine deaminase had little effect itself on the slices, and following the application of DHPG (10 μM), the response to CPA was reduced significantly ($P < 0.05$; $n = 4$) in the maintained presence of the enzyme (Figure 7a). The ability of this deaminase activity to remove adenosine was confirmed by showing its prevention of the responses to adenosine in separate experiments (Figure 7c,d).

The possible involvement of adenosine A_{2A} receptors was then examined by including the selective antagonist ZM241385. When superfused at a concentration of 50 nM, which should be selective for the A_{2A} receptor subtype, this agent had no effect of its own on potential size and did not modify the depression of EPSP slope or paired-pulse inhibition induced by adenosine or DHPG (Figure 8a,b). Following the application of DHPG, however, adenosine responses were

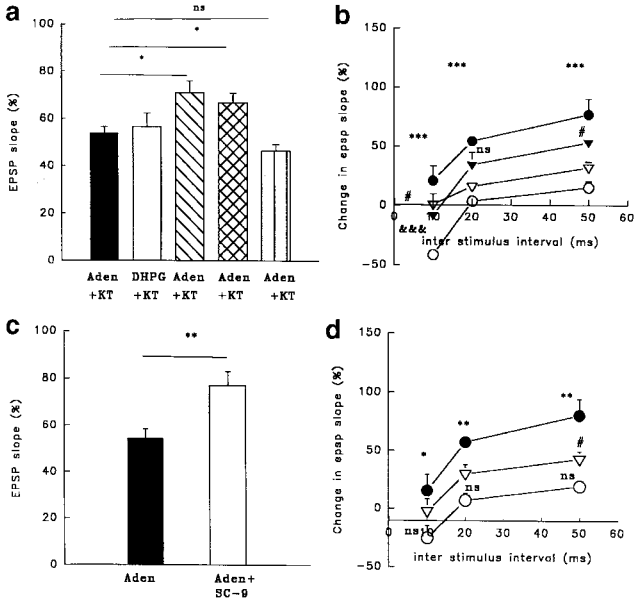

Figure 6 (a) Histogram showing the influence of KT5720 (100 nM) on the interaction between adenosine (10 μM) and DHPG (10 μM) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the effect of adenosine in the presence of KT5720, DHPG in the presence of KT5720, and the responses to adenosine observed 20, 40 and 60 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n=4$). *$P<0.05$. (b) Graph showing the changes of paired-pulse responses. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, adenosine in the presence of KT5720, DHPG in the presence of KT5720 and adenosine 10 μM following DHPG. Only the first adenosine response after DHPG, obtained at 20 min, is shown for clarity. Each point shows the mean ± s.e.m. for $n=4$ slices. ***$P<0.001$ between controls and adenosine before DHPG; #$P<0.05$ between adenosine in the presence and absence of KT5720; &&& $P<0.001$ between controls and DHPG + KT5720 (ANOVA followed by Student–Newman–Keuls test). (c) Histogram showing the influence of SC-9 (10 μM) on the interaction between adenosine (10 μM) and DHPG (10 μM) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of adenosine alone and in the presence of SC-9. The columns indicate the mean ± s.e.m. ($n=4$). **$P<0.01$ (Student's t-test). (d) Graph showing the changes of paired-pulse response size. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of adenosine alone and adenosine in the presence of SC-9. Each point shows the mean ± s.e.m. for $n=4$ slices. *$P<0.05$ and **$P<0.01$ between controls and adenosine before SC-9; #$P<0.05$ between adenosine before and after SC-9 (ANOVA followed by Student–Newman–Keuls test).

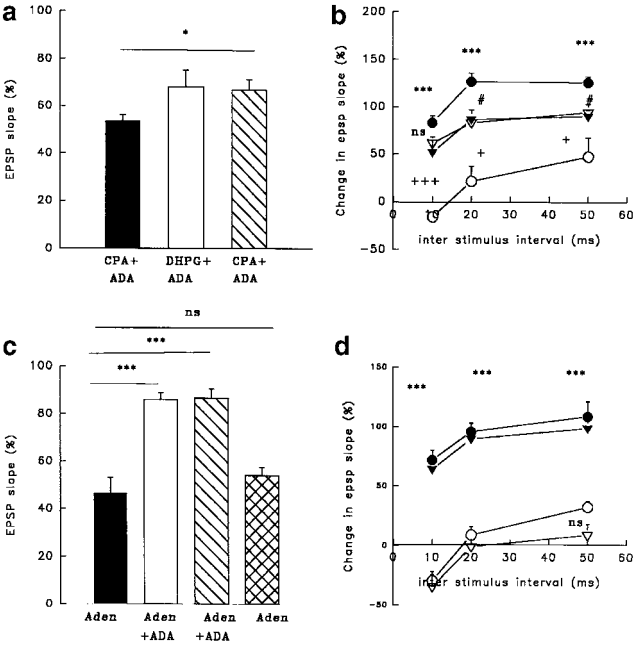

Figure 7 (a) Histogram showing the interaction between CPA (20 nM) and DHPG (10 μM) in the presence of adenosine deaminase (0.1 U ml^{-1}) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of CPA in the presence of adenosine deaminase (CPA + ADA), the effect of DHPG in the presence of adenosine deaminase and the reduced response to CPA observed 20 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n=4$). Statistically significant differences between columns are indicated as *$P<0.05$ (ANOVA followed by Student–Newman–Keuls test). (b) Graph showing the changes of paired-pulse response size. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of CPA 20 nM, DHPG 10 μM and CPA 20 nM following DHPG. Each point shows the mean ± s.e.m. for $n=4$ slices. ***$P<0.001$ between controls and CPA before DHPG; #$P<0.05$ between CPA before and after DHPG (ANOVA followed by Student–Newman–Keuls test). (c) Histogram showing responses to adenosine and the prevention of these responses in the presence of adenosine deaminase 0.1 U ml^{-1} (ADA) present during the second and third applications. The columns indicate the mean ± s.e.m. ($n=4$). Statistically significant differences between columns are indicated as ***$P<0.001$ (ANOVA followed by Student–Newman–Keuls test). (d) Graph showing the changes of paired-pulse response size. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of adenosine before, during and after superfusion with ADA. Each point shows the mean ± s.e.m. for $n=4$ slices. ***$P<0.001$ (ANOVA followed by Student–Newman–Keuls test).

still reduced compared with their initial level, indicating that 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385) had not modified the antagonistic effect of DHPG.

Role of NMDA receptors

In order to examine the possible involvement of NMDA receptors in the interactions between adenosine and metabotropic agonists, the experiments were repeated in the presence of D-2-amino-5-phosphono-pentanoic acid (2AP5), 50 μM. This compound had no effect itself on the slice sensitivity to adenosine, but it prevented the suppression of adenosine responses after perfusion with DHPG, assessed both as the change in EPSP slope and as the change of paired-pulse inhibition (Figure 8c,d).

Discussion

Adenosine acts on presynaptic A_1 receptors to depress neurotransmitter release, and thus to depress transmission in the Schaffer collateral to CA1 synapses. Several previous studies have examined interactions between the ionotropic NMDA receptors and adenosine receptors (Bartrup & Stone, 1990; de Mendonca *et al*., 1995; Norenberg *et al*., 1997; Nikbakht & Stone, 2001), but interactions with mGluRs have received relatively limited attention. Activation of mGluR2

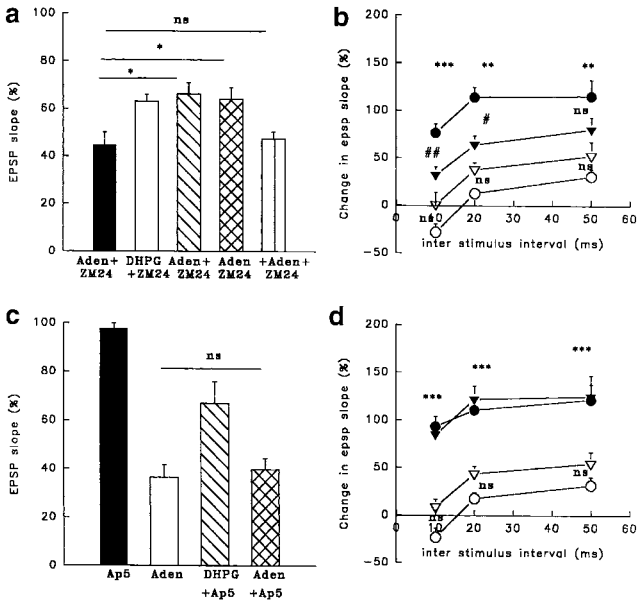

Figure 8 (a) Histogram showing the interaction between adenosine (10 μM) and DHPG (10 μM) in the presence of ZM241385 (50 nM) on the EPSP slope in hippocampal slices. The columns summarise, respectively, the inhibitory effect of adenosine in the presence of ZM241385 (Aden + ZM24), the effect of DHPG in the presence of ZM241385, and the reduced responses to adenosine observed 20 and 40 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n = 4$). Statistically significant differences between columns are indicated as $^{*}P < 0.05$ (ANOVA followed by Student–Newman–Keuls test). (b) Graph showing the changes of paired-pulse response size. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of adenosine 10 μM in the presence of ZM241385, DHPG 10 μM in the presence of ZM241385 and adenosine 10 μM following DHPG. Only the first adenosine response after DHPG, obtained at 20 min, is shown for clarity. Each point shows the mean ± s.e.m. for $n = 4$ slices. $^{***}P < 0.001$ and $^{**}P < 0.01$ between controls and adenosine before DHPG in the presence of ZM241385; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ between adenosine before and after DHPG in the presence of ZM241385 (ANOVA followed by Student–Newman–Keuls test). (c) Histogram showing the interaction between adenosine 10 μM and DHPG 10 μM in the presence of 2AP5 on the EPSP slope in hippocampal slices. The columns summarise, respectively, the lack of effect of 2AP5 alone, the inhibitory effect of adenosine alone (Aden), the effect of DHPG in the presence of 2AP5 and the normal response to adenosine observed 20 min after the application of DHPG. The columns indicate the mean ± s.e.m. ($n = 4$). (d) Graph showing the changes of paired-pulse response size. Data are shown for interstimulus intervals of 10, 20 and 50 ms in control slices, and the effects of adenosine 10 μM in the presence of 2AP5, DHPG 10 μM in the presence of 2AP5 and adenosine 10 μM following DHPG. Each point shows the mean ± s.e.m. for $n = 4$ slices. $^{***}P < 0.001$ between controls and adenosine before DHPG (ANOVA followed by Student–Newman–Keuls test).

receptors reduces the release of adenosine from brain slices (Casabona *et al*., 1994), and the nonselective agonist ACPD inhibits the reduction by adenosine of glutamate release from cortical synaptosomes (Budd & Nicholls, 1995). mGluRs can also potentiate the activation of adenylate cyclase by adenosine A_{2A} receptors (Alexander *et al*., 1992; Schoepp & Johnson, 1993; Winder & Conn, 1993). In a previous electrophysiological study, it was noted that DHPG could suppress the inhibitory activity of the selective adenosine A1 receptor agonist CPA in hippocampal slices (de Mendonca & Ribeiro, 1997), and evidence was obtained using chelerythrine to suggest that this effect was mediated via PKC.

Clarification of the adenosine receptor

In the present study, we have confirmed that ACPD and the group I receptor agonist DHPG depress the inhibitory effects of adenosine and CPA (de Mendonca & Ribeiro, 1997). In principle, however, this apparent antagonism could result from an inhibition of A_1 receptor responses, or a facilitation of adenosine A_{2A} receptor responses generated by endogenous levels of adenosine. For example, a synergistic interaction has been described between adenosine A_{2A} receptors and mGluRs in elevating cyclic AMP levels (Alexander *et al*., 1992; Schoepp & Johnson, 1993; Winder & Conn, 1993). In the present work, we have removed endogenous adenosine with adenosine deaminase, allowing us to conclude that the interaction is between group I glutamate receptors and adenosine A_1 receptors. We have also obtained further evidence in support of this conclusion, with the demonstration that the A_{2A} receptor blocker ZM241385 did not modify the ability of DHPG to suppress adenosine sensitivity.

Clarification of the metabotropic receptor

In the present work, both ACPD and DHPG produced a significant depression of synaptic potentials. Inhibitory effects of ACPD on synaptic transmission have been reported in several brain regions and across species (King & Liu, 1996; Krieger *et al*., 1996), including inhibition of synaptic transmission at the Schaffer collateral–CA1 synapse (Gereau & Conn, 1995). Both subtypes of group I receptor ($mGluR_1$ and $mGluR_5$) are known to be present in the hippocampal CA1 region. Many effects of group I agonists have been attributed to the $mGluR_5$ subtype, largely because of its presence in higher abundance in the CA1 region (Lujan *et al*., 1996). Immunocytochemical studies have revealed that group I mGluRs are located primarily on the postsynaptic sites, rather than presynaptically in the CA1 area (Lujan *et al*., 1996; Shigemoto *et al*., 1997). However, functional evidence has indicated the presence of presynaptic group I mGluRs that increase intraterminal calcium levels and promote transmitter release (Schwartz & Alford, 2000; Croucher *et al*., 2001).

While the use of selective ligands makes it clear that group I receptors are responsible for the interaction with adenosine (this paper and de Mendonca & Ribeiro, 1997), we have now extended this observation by showing that the effects of DHPG upon adenosine sensitivity could be prevented by LY367385, an antagonist selective for the $mGlu_{1a}$ subtype (Mannaioni *et al*., 2001), but not by SIB1893, which is selective for the $mGlu_5$ subtype (Varney *et al*., 1999). At the concentrations used here, both LY367385 and SIB1893 have been shown to produce a greater than 50% blockade of responses mediated by $mGlu_{1a}$ and $mGlu_5$ receptors in other experimental paradigms (Varney *et al*., 1999; Mannaioni *et al*., 2001). We conclude that it is primarily the $mGlu_{1a}$ subtype that mediates the suppression of adenosine sensitivity.

Time course

In addition, it is apparent that the interaction between glutamate and adenosine receptors can last for up to at least 60 min after a 10 min application of ACPD or DHPG. This is consistent with biochemical studies that have reported effects of group I metabotropic receptor stimulation lasting up to

40 min (Alexander *et al*., 1992; Budd & Nicholls, 1998), and changes of single cell excitability lasting up to 60 min (Budai & Larson, 1998). Even transient increases in the extracellular levels of glutamate could produce long-lasting changes of adenosine sensitivity.

The long time course of action of ACPD and DHPG would also be consistent with the apparent mediation of the metabotropic receptor effects by PKC. De Mendonca & Ribeiro (1997) obtained evidence for this using the kinase inhibitor chelerythrine, a result that we have confirmed here. In addition, we have shown that the more selective PKC inhibitor GF109203X can also prevent the effects of DHPG, and a reduction of adenosine sensitivity can be reproduced by the PKC activator 5-chloro-*N*-(6-phenylhexyl)-1-naphthalenesulphonamide (SC-9). In contrast, inhibition of PKA had no effect on responses to DHPG. A role for PKC would be entirely consistent with previous demonstrations that the activation is able to decrease the presynaptic inhibitory effects of adenosine in cerebrocortical synaptosomes (Budd & Nicholls, 1995), the hippocampus (Thompson *et al*., 1992) and neuromuscular junction (Sebastiao *et al*., 1990).

Role of NMDA receptors

Activation of group I mGluRs can facilitate the activation of NMDA receptors (Fitzjohn *et al*., 1996; Awad *et al*., 2000; Salt & Binns, 2000; Attucci *et al*., 2001), probably mediated by PKC (Pisani *et al*., 1997; Liao *et al*., 2001). Since we have previously shown that activation of NMDA receptors can reduce the inhibitory activity of adenosine (Bartrup & Stone, 1990; Nikbakht & Stone, 2001), it was possible that the metabotropic receptor-mediated reduction of adenosine sensitivity was mediated indirectly by a facilitation of NMDA receptor activity. The results do indeed indicate that the mGluR/adenosine interaction could be prevented by the NMDA antagonist 2AP5, making this a possible explanation.

Presynaptic mechanism and nonselectivity

An important finding in the present study is that the depressant effect of group I receptor agonists is not selective for adenosine A_1 receptors, but is also exerted against the $GABA_B$ receptor agonist baclofen. This raises the possibility that the group I effects may not result from interactions at the receptor level, but from generalised changes in presynaptic terminal excitability or release processes, probably mediated via PKC. Indeed, although relatively little work has been performed on functional aspects of group I metabotropic receptors directly on synaptic terminals, Schwartz & Alford (2000) and Croucher *et al*. (2001) have concluded that group I receptors on presynaptic terminals can increase glutamate release.

The preceding discussion is based on the assumption that the interactions between the group I receptor agonists and adenosine or baclofen are mediated at a common, presumably presynaptic, site. Our use of paired-pulse interactions confirms that this is so. Particularly when studied on EPSPs, the paired-pulse paradigm represents a valuable means of assessing the actions of compounds on presynaptic terminals. The paired-pulse inhibition obtained at interpulse intervals of 10 ms results from the depletion of transmitter from presynaptic stores (Burke & Hablitz, 1994; Wilcox & Dichter, 1994; Hashimoto & Kano, 1998), and is reduced by agents or procedures that decrease transmitter release. Paired-pulse facilitation, observed at longer interpulse intervals, is because of the residual intraterminal Ca^{2+}, which increases transmitter release (Hess *et al*., 1987; Wu & Saggau, 1994; Debanne *et al*., 1996; Kleschevnikov *et al*., 1997). The fact that the mGluR agonists can suppress the effects of adenosine in this system provides a strong argument that the interactions are occurring at presynaptic terminals. The present data do not, however, address the question as to whether the interaction between group I receptors and adenosine is a direct or indirect action. There is clear evidence that the activation of group I metabotropic receptors at postsynaptic sites can lead to the release of a retrograde messenger that is responsible for at least part of the presynaptic effects of agonists (Levenes *et al*., 2001; Maejima *et al*., 2001; Watabe *et al*., 2002). Only when there is a clear identification of the nature of such a messenger can its role in the depression of adenosine sensitivity be investigated further.

AS thanks the Iranian Ministry of Education for support.

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(Received September 6, 2002
Accepted November 4, 2002)