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Suppression of presynaptic responses to adenosine by activation of NMDA receptors

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Abstract

The interactions between adenosine and NMDA receptors has been investigated using the paired-pulse paradigm in hippocampal slices. This technique allows the study of drug effects specifically at presynaptic terminals. The inhibitory effect of adenosine on population spikes, and the decrease of paired-pulse inhibition assessed using either population spikes or population excitatory postsynaptic potentials, were suppressed by performing the experiments in magnesium-free medium, or by superfusion of the slices with *N*-methyl-D-aspartate (NMDA) at a concentration (4 μM) which did not itself affect potential size. The suppressant effect of NMDA was prevented by 2-amino-5-phosphonopentanoic acid. All these interactions were still seen in the presence of bicuculline methobromide, 30 μM. Neither α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) nor kainate produced a suppression of adenosine responses. The presence of NMDA did not modify the effects of baclofen on population potentials or paired-pulse inhibition. Activating NMDA receptors by the induction of long-term potentiation or by superfusion with glycine also reduced significantly the effects of adenosine on population spikes and paired-pulse interactions. Increasing population potential size by a mechanism which did not involve the activation of NMDA receptors (increasing stimulus strength) did not change sensitivity to adenosine. When adenosine receptor-selective agonists were tested, it was found that NMDA did not modify the inhibitory effect of the adenosine A_1 receptor agonist N^6-cyclopentyladenosine, but did enhance the excitatory effect of the adenosine A_{2A} receptor agonist 2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680). The combined response to NMDA and CGS21680 was prevented by the adenosine A_{2A} receptor selective antagonist 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385). It is concluded that NMDA receptor activation can suppress neuronal sensitivity to adenosine by acting at presynaptic sites, and that this interaction results from an increase in the excitatory action of adenosine A_{2A} receptors, rather than a depression of A_1 receptor function.

Keywords: Adenosine; Glutamate; NMDA receptor; NMDA (*N*-methyl-D-aspartate); Presynaptic receptor

1. Introduction

Adenosine acts on four types of receptor, known as A_1, A_{2A}, A_{2B} and A_3 receptors, in a variety of tissues including the central nervous system (CNS). Understanding the effects and neurochemical modulatory influences on these receptors is of growing importance in view of the developing interest in antagonists at adenosine A_1 receptors as potential cognitive enhancers (Schingnitz et al., 1991; Suzuki et al., 1993; Spedding and Williams, 1996), and of adenosine A_{2A} receptor antagonists as potential neuroprotective agents (Jones et al., 1998a,b; Ongini et al., 1997).

In 1988, we reported that the presynaptic inhibitory effects of adenosine on glutamate release in the hippocampal CA1 region were dependent on the presence of magnesium, since removal of this ion from the superfusing medium prevented responses to adenosine (Bartrup and Stone, 1988). This change was later shown to be reproduced by superfusing *N*-methyl-D-aspartate (NMDA), and prevented by including blockers of the NMDA-sensitive receptors (such as dizocilpine or 2-amino-5-phosphonopentanoic acid) before the removal of magnesium (Bartrup and Stone, 1990). This result suggested that activation of NMDA receptors was involved in the suppression of adenosine sensitivity. Also consistent with this view was the weaker ability of adenosine receptor activation to suppress neuronal firing induced by microiontophoretically applied NMDA compared with firing induced by acetylcholine or quisqualate (Bartrup et al., 1991).

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There remains a major question as to the site of the adenosine/NMDA interaction—presynaptic or postsynaptic. We have therefore revisited the above findings by extending the previous work in several directions. Firstly, we have re-examined the effects of adenosine/NMDA combinations using the paired-pulse paradigm, which is widely accepted as providing a more accurate indication of presynaptic events than the study of population spikes and postsynaptic potentials (Hess et al., 1987; Wilcox and Dichter, 1994; Wu and Saggau, 1994; Hashimoto and Kano, 1998). Secondly, we have examined the effects of glycine and long-term potentiation as alternative methods of activating NMDA receptors. Thirdly, we have examined the selectivity of the NMDA/adenosine interaction by studying the effects of NMDA receptor activation on responses to the $GABA_B$ receptor agonist baclofen. Finally, we have investigated the adenosine receptor subtype selectivity of the NMDA interaction.

2. Methods

Hippocampal slices were obtained from male Wistar rats as described previously (Higgins and Stone, 1996;

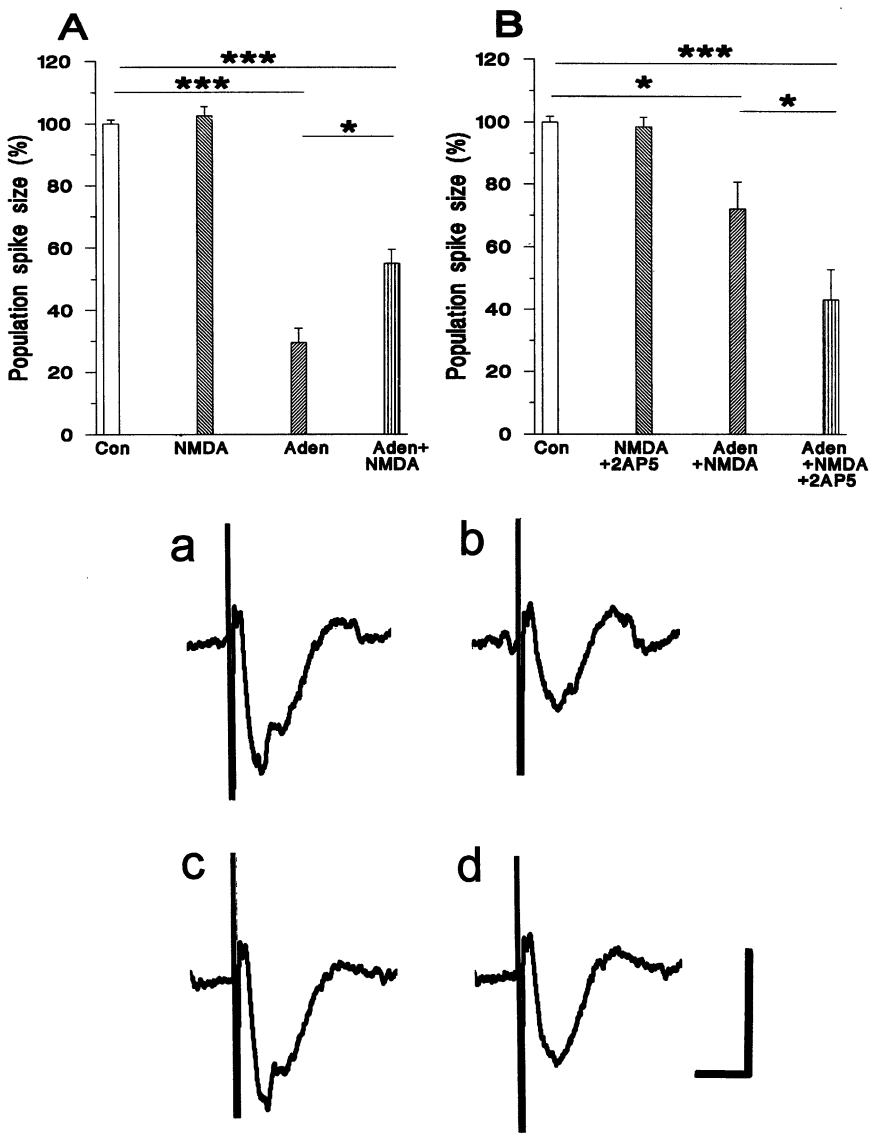

Fig. 1. Histograms summarizing the interactions between adenosine and NMDA on population spike potentials in hippocampal slices. (A) Summarises the amplitude of control spikes expressed as 100% (Con), the lack of effect of NMDA alone at 4 μM (NMDA), the inhibitory effect of adenosine, 10 μM (Aden) and adenosine in the presence of NMDA, 4 μM. (B) Summarises the amplitude of control spikes expressed as 100% (Con), the lack of change seen in NMDA 4 μM plus 2AP5 50 μM, the inhibitory effect of adenosine 10 μM in the presence of NMDA (4 μM) and adenosine in the presence of NMDA and 2AP5 (50 μM). The columns indicate the mean ± S.E.M. ($n = 4$ for (A) and $n = 5$ for (B)). Statistically significant differences between columns are indicated as $^{*}P < 0.05$, $^{***}P < 0.001$. The original records below illustrate these results using field EPSPs. (a) is a control EPSP, (b) the effect of adenosine 10 μM. Record (c) is taken in the presence of NMDA 4 μM, and (d) shows the reduced response to adenosine in the presence of NMDA. Calibrations: 1 mV and 5 ms.

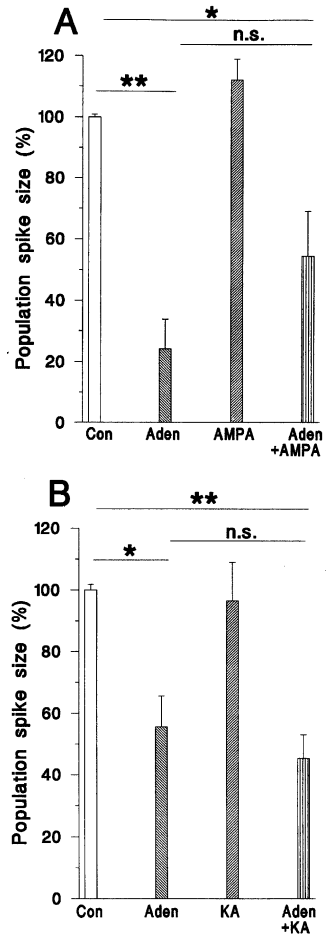

Fig. 2. Histograms summarizing the interactions between adenosine and glutamate receptor agonists on population spike potentials in hippocampal slices. (A) Summarises the amplitude of control spikes expressed as 100% (Con), the inhibitory effect of adenosine, 10 μM (Aden), the effect of AMPA 100 nM (AMPA) and the effect of adenosine in the presence of AMPA. (B) Summarises the amplitude of control spikes expressed as 100% (Con), the inhibitory effect of adenosine, 10 μM (Aden), the effect of kainate 100 nM (KA) and adenosine in the presence of kainate. The columns indicate the mean ± S.E.M. ($n = 4$ for (A) and $n = 3$ for (B)). Statistically significant differences between columns are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$.

Nikbakht and Stone, 2000). Briefly, animals were anaesthetised with urethane (1.5 g kg^{-1}, i.p.) and killed by cervical dislocation. The brains were rapidly taken out and placed in cold artificial cerebrospinal fluid (ACSF) of composition of (mM) KH_2PO_4 2.2; KCl 2.0; $NaHCO_3$ 25; NaCl 115; $CaCl_2$ 2.5; $MgSO_4$ 1.2 and glucose 10, saturated with 95% oxygen–5% carbon dioxide. Both hippocampi were dissected out and transverse slices, 450 μM thick, were prepared using a McIIwain tissue chopper. The slices were maintained at room temperature in an interface chamber containing ACSF gassed with 95% O_2–5% CO_2. Electrophysiological recordings were started at least 1 h after slice preparation. Individual slices were transferred to a 1-ml chamber, which was continually perfused with ACSF at a rate of 3–4 ml min^{-1}. Extracellular evoked population spikes were recorded from pyramidal cell bodies using glass microelectrodes (1 M NaCl, resistances approximately 2–5 MΩ) following stimulation of the

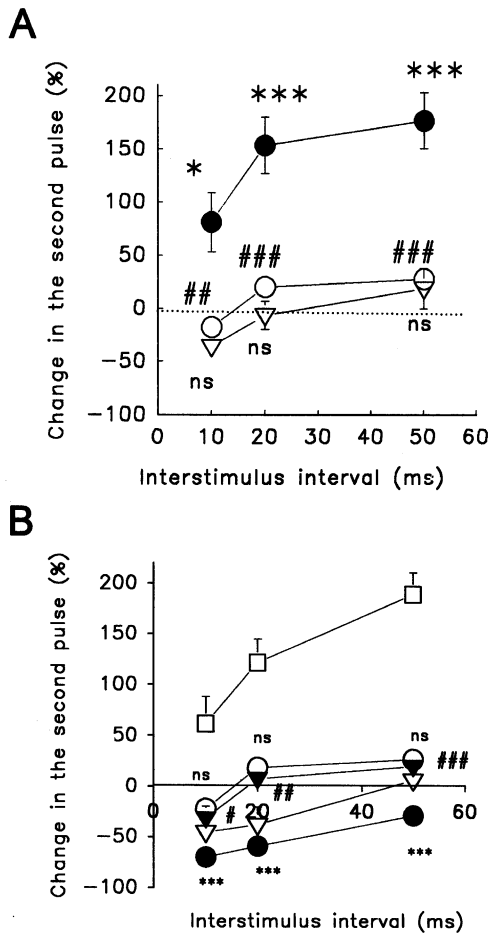

Fig. 3. The effect of agents on paired-pulse interactions. (A) Control slices (open circles) exhibit paired-pulse inhibition at an interstimulus interval of 10 ms, with facilitation at intervals of 20 and 50 ms. The size of the second evoked potential compared with the first is shown for control slices (open circles), adenosine 10 μM (closed circles) and adenosine in magnesium-free ACSF (open triangles). Each point shows the mean ± S.E.M. (where greater than symbol size), with significance denoted between controls and adenosine as $^{*}P < 0.05$, $^{***}P < 0.001$, and between adenosine and adenosine in magnesium-free medium as $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ ($n = 3$). There were no significant differences between the data in magnesium-free medium and the control curve. (B) Illustrates the blockade of the magnesium-free effect by 2AP5. The size of the second evoked potential compared with the first is shown for control slices (open circles), and control responses in magnesium-free medium (closed circles). Potentials recorded in magnesium-free medium with 2AP5 (closed triangles) are restored to the original control levels. Adenosine 10 μM in magnesium-free medium (open triangles) has no effect on paired-pulse potentials, whereas in the presence of 2AP5 in magnesium-free medium, adenosine responses are clearly observed (open squares) and are comparable to those seen in normal ACSF medium (see closed circles in graph A). Each point shows the mean ± S.E.M. (where greater than symbol size), with significance between controls and magnesium-free medium denoted as $^{***}P < 0.001$, and between magnesium-free medium with and without 2AP5 denoted by $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ ($n = 5$). There were no significant differences between data in magnesium-free medium and ACSF.

Schaffer collateral and commissural fibres in stratum radiatum. Stimulation was achieved using a concentric bipolar electrode (Clark Electromedical, Havard Apparatus) with pulses of 50–300 μs duration delivered at 0.1 Hz. Evoked responses were amplified, and a computer was used to digitize, analyse and store the responses via a CED (Cambridge Electronic Design) micro1401 interface. Slices were used only when the maximum population spike was at least 3 mV. Following the optimal placement of the electrodes, the intensity of stimulation was adjusted to yield an evoked potential which was approximately 70% of maximum so as to allow increases or decreases of potential size to be detected. The effect of each drug was assessed by measuring any change in the size of the population spike measured, in mV, from the mid-point between the two peaks of the positive-going synaptic potential and the peak of the negative population spike.

For paired-pulse recording, the intensity of the conditioning stimulus (first pulse) and testing stimulus (second pulse) were maintained the same. Interpulse intervals of 10, 20 and 50 ms were used as in previous studies (Higgins and Stone, 1993, 1996; Nikbakht and Stone, 2000). When paired-pulse interaction was examined, inhibition or facilitation was expressed as the percentage change in the response to the second stimulus compared with the first. Comparisons of data between the control and the drug perfused groups were made using a paired or unpaired *t*-test, or analysis of variance (ANOVA) for groups of data as appropriate. The significance level was set at $P < 0.05$.

Adenosine, N^6-cyclopentyladenosine, baclofen, kainic acid and glycine were obtained from the Sigma. 2-Amino-5-phosphonopentanoic acid (2AP5), NMDA, 5,7-dichlorokynurenic acid, bicuculline methochloride, α-amino-3-

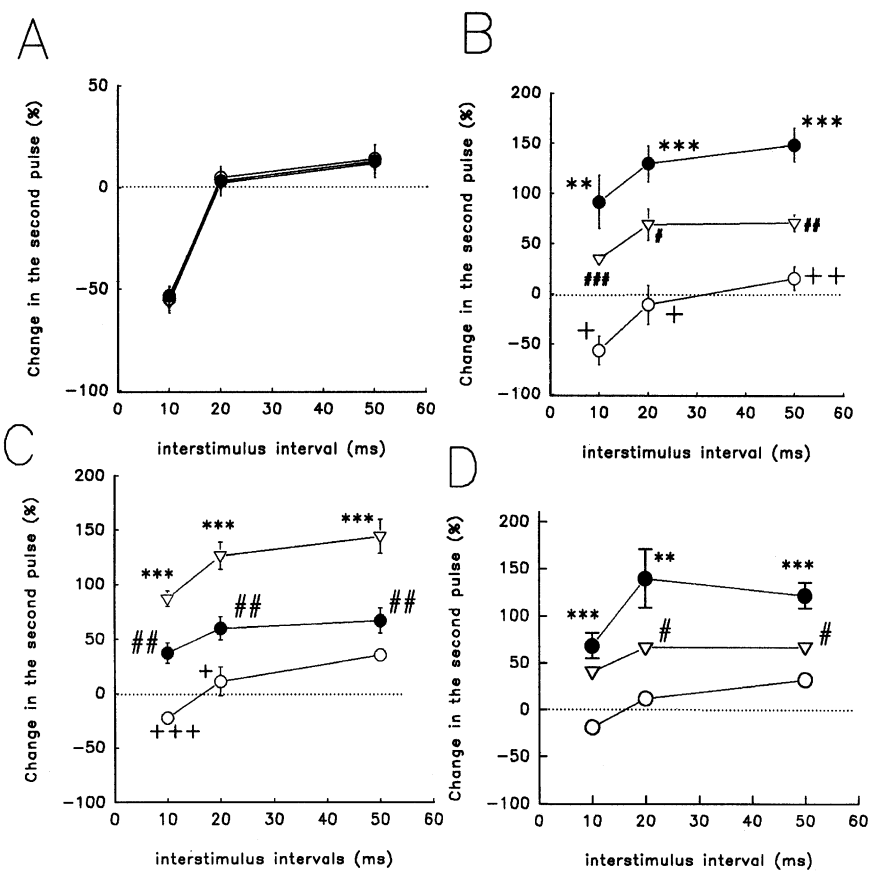

Fig. 4. The effect of agents on paired-pulse interactions. (A) The size of the second evoked potential compared with the first is shown for control slices (open circles), the effect of NMDA 4 μM (closed circles) and after washing for 30 min (open triangles). Each point indicates the mean, with S.E.M. being less than the symbol size ($n = 4$). (B) Summarises data from control slices (open circles), the effects of adenosine 10 μM (closed circles) and adenosine in the presence of NMDA (open triangles). Each point shows the mean ± S.E.M. ($n = 5$), with significant differences indicated between controls and adenosine ($^{**}P < 0.01$, $^{***}P < 0.001$), between controls and NMDA plus adenosine ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$) and between adenosine and adenosine with NMDA ($^{+}P < 0.05$, $^{++}P < 0.01$). (C) Illustrates control slices (open circles), adenosine in the presence of NMDA (closed circles) and adenosine in the presence of NMDA and 2AP5 50 μM (open triangles). Each point shows the mean ± S.E.M. ($n = 4$), with significant differences indicated between controls and NMDA plus adenosine ($^{+}P < 0.05$, $^{+++}P < 0.001$), between controls and adenosine with NMDA and 2AP5 ($^{***}P < 0.001$), and between adenosine plus NMDA with and without 2AP5 ($^{\#\#}P < 0.01$). (D) Demonstrates that the same interaction was observed when paired-pulse tests were performed using the population EPSP. The data shown are from control slices (open circles), the effects of adenosine 10 μM (closed circles) and adenosine in the presence of NMDA (open triangles). Each point shows the mean ± S.E.M. ($n = 5$), with significant differences indicated between controls and adenosine ($^{**}P < 0.01$, $^{***}P < 0.001$), and between adenosine and adenosine with NMDA ($^{\#}P < 0.05$).

hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and 2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680) were obtained from Research Biochemicals, 4-(2-[7-amino-2-(2-furyl) [1,2,4]triazolo[2,3a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385) was obtained from Tocris Chemicals.

3. Results

3.1. Population potentials

The effects of NMDA itself upon the size of individual population spikes were concentration-dependent. At 4 μM, no change was detected in spike size, whereas 6 μM induced a decrease of spike size of $22 \pm 15.3\%$ ($n = 4$) compared with the initial baseline potential, with a short-lasting increase in amplitude reaching $17.3 \pm 3.1\%$ at its peak occurring on washout. At 10 μM NMDA, a larger depression was obtained of $54.8 \pm 8.8\%$ ($n = 4$) with a larger but inconsistent increase on washout. The subthreshold concentration of 4 μM was therefore selected for the subsequent experiments, so that directly induced changes of potential size could not complicate the interpretation of responses to adenosine.

A concentration of 10 μM adenosine was selected for study as previous work has shown that this consistently yields an approximately 60–70% depression of evoked potentials, allowing either an increase or decrease of response size to be detected. In this first series of experiments, adenosine at 10 μM depressed the population spike amplitude by $70.6 \pm 5.0\%$ of the control size (Fig. 1A).

The superfusion of NMDA at 4 μM for 10 min before and during the application of adenosine 10 μM resulted in a significant suppression of the responses to adenosine, with the latter now producing a decrease of spike size of only $45.0 \pm 4.8\%$ (Fig. 1A) ($P < 0.05$; $n = 4$). The inclusion of 2AP5 (50 μM) prevented this suppression of adenosine responses by NMDA. Thus, in a different series of slices, the responses to adenosine were larger in the presence of NMDA and 2AP5, than in the presence of NMDA alone ($P < 0.05$; $n = 5$); responses to adenosine were restored to the same size as control responses to adenosine alone (Fig. 1B).

3.2. AMPA and kainic acid

At concentrations above 100 nM, both AMPA and kainic acid produced a depression of population spike amplitude. The subthreshold concentration of 100 nM was therefore selected to test in combination with adenosine in order to examine the specificity of the interaction with NMDA. However, when examined on population spikes, neither compound was able to change significantly the depressant effect of adenosine 10 μM (Fig. 2).

3.3. Paired-pulse interactions

At interpulse intervals of 10, 20 and 50 ms, paired-pulse stimulation induced the pattern of early paired-pulse inhibition and later facilitation reported previously (Higgins and Stone, 1996; Nikbakht and Stone, 2000) and indicated in Fig. 3. Adenosine at 10 μM decreased the size of the inhibitory component at a 10-ms interstimulus interval and increased the facilitation at later intervals (Fig. 3). When the paired-pulse interaction was examined in magnesium-free ACSF, the depressant response to adenosine was no

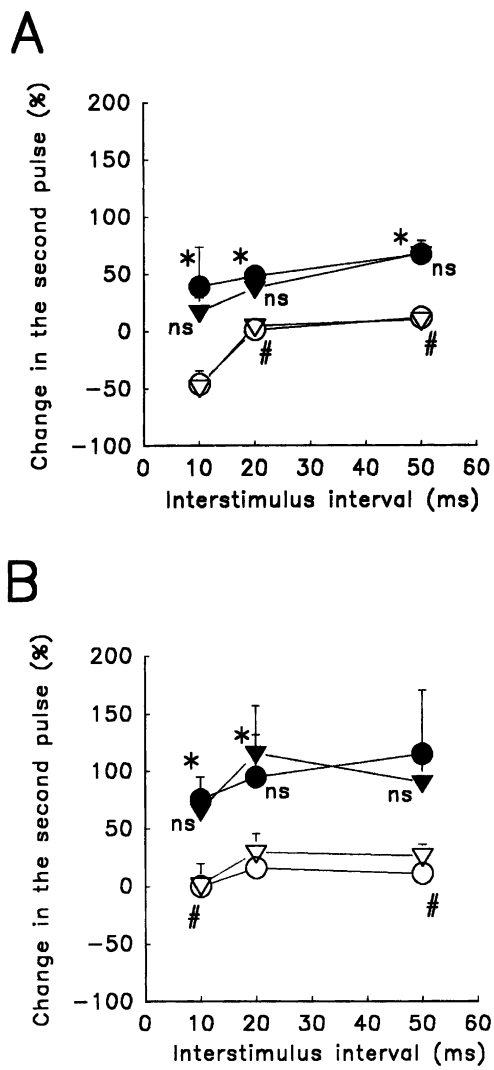

Fig. 5. The effect of agents on paired-pulse interactions. (A) The size of the second evoked potential compared with the first is shown for control slices (open circles), adenosine 10 μM (closed circles), AMPA 100 nM (open triangles) and adenosine plus AMPA (closed triangles). (B) Summarises data from control slices (open circles), the effects of adenosine 10 μM (closed circles), kainate 100 nM (open triangles) and adenosine in the presence of kainate (closed triangles). Each point shows the mean ± S.E.M., with significant differences indicated between controls and adenosine ($^{*}P < 0.05$; $n = 3$) and between controls and adenosine with AMPA or kainate ($^{\#}P < 0.05$; $n = 3$). There were no significant differences between the effects of adenosine alone or in the presence of AMPA or kainate.

longer observed (Fig. 3). In order to determine whether this loss of adenosine sensitivity was related to the presumed activation of NMDA receptors in magnesium-free media, this experiment was repeated in the presence of 2AP5. As shown in Fig. 3B, this antagonist prevented the increased paired-pulse inhibition produced in magnesium-free medium, and also restored the ability of adenosine to reduce paired-pulse inhibition and to increase facilitation.

NMDA itself, at the concentration of 4 μM which did not directly affect population potentials, did not modify paired-pulse interactions (Fig. 4A). However, when perfused together with adenosine at 10 μM, this concentration of NMDA was sufficient to reduce significantly the effects of adenosine on paired-pulse phenomena (Fig. 4B). The inclusion of 2AP5 50 μM prevented this suppressant action of NMDA and increased significantly the size of paired-pulse facilitation (Fig. 4C).

The interaction between NMDA and adenosine was still observed when paired-pulse experiments were performed using population excitatory postsynaptic potentials rather than population spikes (Fig. 4D). Similarly, the interactions between NMDA and adenosine were unchanged when experiments were performed in the presence of bicuculline methobromide, 30 μM, to exclude the involvement of receptors located on interneurones.

As illustrated in Fig. 5, neither AMPA (Fig. 5A) nor kainic acid (Fig. 5B) produced any effect on paired-pulse inhibition at 100 nM, and neither compound produced any change in the ability of adenosine to reduce paired-pulse inhibition or to enhance paired-pulse facilitation.

3.4. Effect of long-term potentiation

As an alternative method of activating NMDA receptors, and one which may be more physiologically relevant than slice perfusion with NMDA itself, long-term potentiation was induced using a train of stimuli delivered at 100 Hz for 1 s. The potentiation reached a level of 130.14 ± 4.09% ($n = 4$) relative to the preceding control potentials. The effects of adenosine on population spikes (Fig. 6A) and paired-pulse interactions (Fig. 6B) compared before and 10 min after the establishment of potentiation. Following the induction of long-term potentiation, the depressant responses to adenosine were significantly reduced (Figs. 6B and 7).

In order to control for the fact that long-term potentiation, by definition, involves an increase in population spike

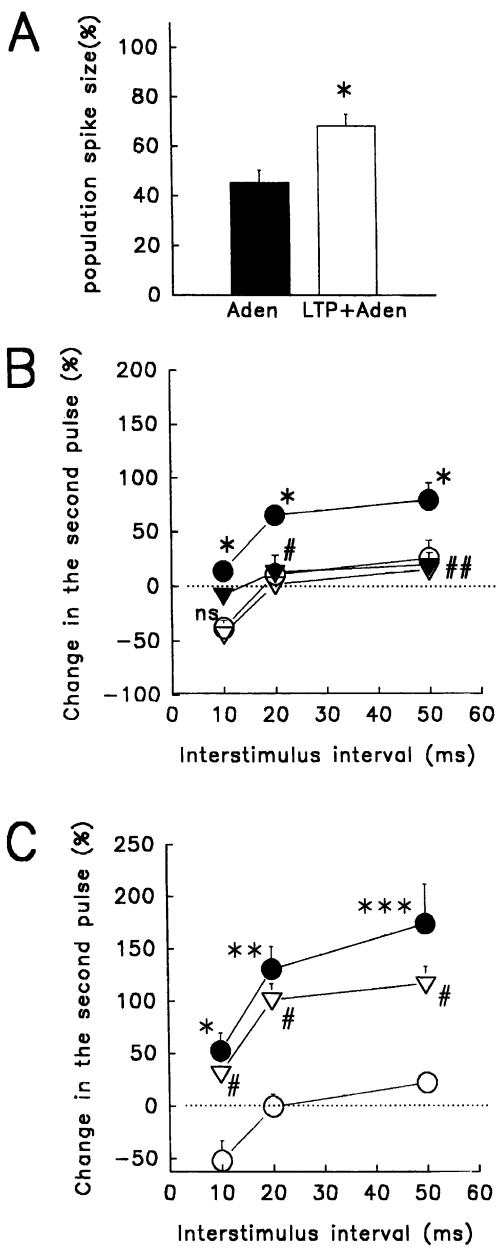

Fig. 6. The influence of electrically induced long-term potentiation (long-term potentiation) on population spikes and paired-pulse interactions. (A) The inhibitory effect of adenosine 10 μM on single population spike size (closed column) is reduced 10 min after the induction of long-term potentiation (open column). $^{*}P < 0.05$ ($n = 5$). (B) The size of the second evoked potential compared with the first is shown for control slices (open circles), adenosine 10 μM (closed circles) and adenosine 10 min after the establishment of long-term potentiation (closed triangles). Long-term potentiation itself had no effect on the paired-pulse interactions (open triangles) ($n = 3$). Each point shows the mean ± S.E.M., with significant differences indicated between controls and adenosine responses ($^{*}P < 0.05$; $n = 3$), and between adenosine reponses before and after the establishment of long-term potentiation ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$; $n = 3$). (C) Summarises data from control slices (open circles), the effects of adenosine 10 μM using the submaximal evoked potentials used routinely in these studies (closed circles) and the effects of adenosine when stimulus strength had been increased to yield a maximal potential size (open triangles). Symbols indicate significance between controls and adenosine responses on submaximal potentials ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) and on maximal potentials ($^{\#}P < 0.05$). There were no significant differences between the responses to adenosine at the different stimulus strengths.

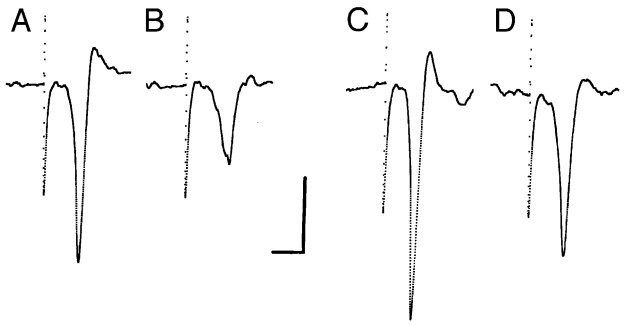

Fig. 7. Sample records of the influence of long-term potentiation on the effects of adenosine on spike potentials. (A) Control potential; (B) reduced potential in the presence of adenosine 10 μM; (C) control potential 10 min after the electrical induction of long-term potentiation; (D) response to adenosine 10 μM tested 10 min after the induction of long-term potentiation. The inhibition by adenosine is significantly reduced after long-term potentiation. Calibrations: 2 mV and 5 ms.

size, experiments were also performed to assess the effect of increasing the stimulus strength from the usual level (producing a spike size approximately 70% of the maximum attainable, see Methods), to a level producing a maximal spike size. While this procedure does no reproduce changes of release probability associated with long-term potentiation, it does act as a useful control for changes of tissue excitability. The manoeuvre did not modify significantly the inhibitory effect of adenosine, as illustrated in Fig. 6C, indicating that the effect of long-term potentiation was not the result of the change in overall excitability of the neurones.

3.5. Glycine

In order to examine the effects of activating NMDA receptors by a method other than the direct activation of the glutamate binding site, we have also used glycine to activate the strychnine-resistant allosteric glycine binding site on the NMDA receptor. When applied alone at 1 mM for 10 min, glycine induced a long-term potentiation of population spike size which attained a level of $120.45 \pm 5.45\%$ ($n = 4$) after 30 min of washing relative to the control potential amplitude (Fig. 8). This potentiation could be prevented by superfusing the slices with 5,7-dichlorokynurenic acid 20 μM or 2AP5 50 μM for 10 min before and during the application of glycine (potentiation of $103.0 \pm 3.5\%$, $n = 4$, and $105.4 \pm 4.3\%$, $n = 4$, respectively), confirming that the glycine-induced long-term potentiation involved the activation of NMDA receptors. At a higher concentration of 5 mM, glycine induced a depression of population spike size, which was followed by long-term potentiation of the potential size which was maintained for at least 30 min (Fig. 8). The similarity of mechanism between electrically and glycine-induced long-term potentiation was indicated by the fact that saturation of the glycine-induced change by repeated applications, was able to occlude long-term potentiation induced electrically (Fig. 8).

The lower concentration of 1 mM glycine was selected for testing in combination with adenosine, so that the direct inhibitory activity of 5 mM would not complicate interpretation. The effect of adenosine on paired-pulse interactions was not changed during the superfusion of glycine, but its effects were reduced significantly when tested 30 min after the application of glycine, a time at which the long-term potentiation by glycine was established (Fig. 9).

3.6. Responses to baclofen

The $GABA_B$ receptor agonist baclofen at 2 μM substantially depressed population spike size (Fig. 10A) and

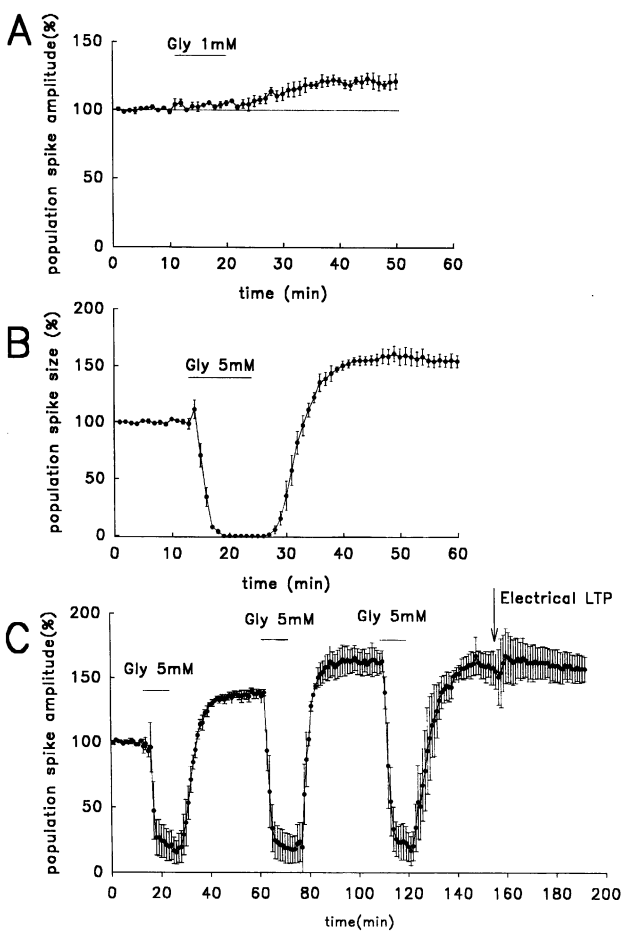

Fig. 8. Changes in population spike size produced by superfusion with (A) glycine 1 mM and (B) glycine 5 mM. In both cases, a long-term potentiation of potential size was induced. (C) Illustrates the effect of applying three successive pulses of glycine which results in a saturation of the glycine-induced long-term potentiation. At that time, electrically induced long-term potentiation is also occluded, suggesting similarity in the two phenomena. Each point shows the mean ± S.E.M. ($n = 4$ in each case).

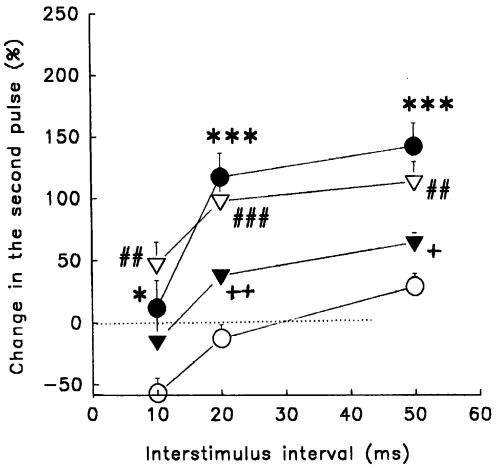

Fig. 9. Graph summarising the effect of agents on paired-pulse interactions. Data are shown from control slices (open circles), the effects of adenosine 10 μM (closed circles), adenosine at the end of a 10-min superfusion of glycine (open triangles) and adenosine 30 min after the glycine perfusion when long-term potentiation has been established (closed triangles). Each point shows the mean ± S.E.M. ($n = 5$), with significant differences indicated between controls and adenosine ($^{*}P < 0.05$, $^{***}P < 0.001$), between controls and adenosine after 10 min of glycine ($^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$), between adenosine alone and adenosine 30 min after glycine perfusion ($^{+}P < 0.05$, $^{++}P < 0.01$). There were no significant differences between control data and the effects of adenosine tested 30 min after glycine.

also reduced paired-pulse inhibition and enhanced facilitation (Fig. 10C). In contrast to the effect of adenosine, this depression was not modified by superfusion with NMDA at 4 μM (Fig. 10B). Similarly, the extent of the depression by baclofen was not reduced by the electrical induction of long-term potentiation (Fig. 10A), and the effects of baclofen on paired-pulse interactions were unchanged in the presence of NMDA (Fig. 10C).

3.7. Adenosine receptor subtype

In order to determine whether the suppressive activity of NMDA receptors was directed selectively towards the adenosine A_1 or A_{2A} receptors, the effects of the adenosine A_1 receptor agonist cyclopentyladenosine (CPA) and the A_{2A} receptor agonist CGS21680 were studied. At a concentration of 50 nM, CPA depressed population spike size by $41.8 \pm 8.4\%$ ($n = 3$), but this effect was not

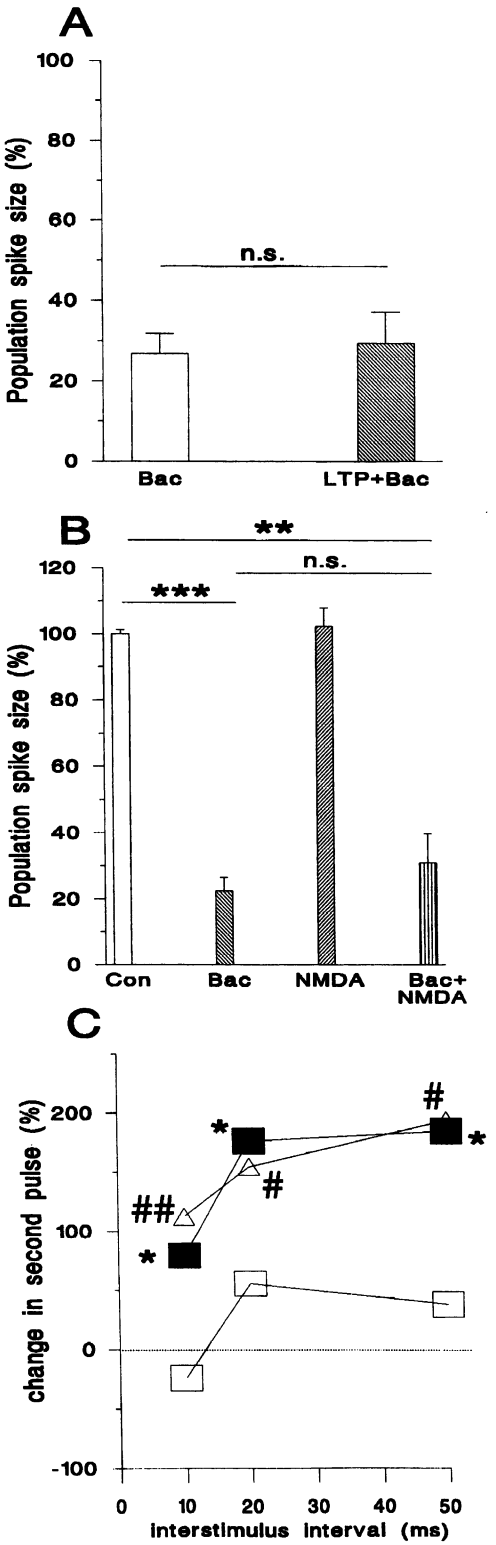

Fig. 10. (A) The depressant activity of baclofen on population spikes (Bac) was not changed following the induction of long-term potentiation (LTP + Bac). (B) The size of population spikes was decreased from control size (Con) by baclofen 2 μM (Bac), but was not affected by NMDA 4 μM applied alone (NMDA). The presence of NMDA did not change the effect of baclofen (Bac + NMDA) ($^{**}P < 0.01$, $^{***}P < 0.001$; $n = 5$). (C) Paired-pulse interactions showing data from control slices (open squares), the effects of baclofen 2 μM (closed squares), and baclofen in the presence of NMDA (open triangles). Each point shows the mean ± S.E.M., with significant differences indicated between controls and baclofen ($^{*}P < 0.05$; $n = 3$) and between controls and baclofen plus NMDA ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$; $n = 3$). There were no significant differences between the effects of baclofen alone and in the presence of NMDA.

changed (depression by $44.6 \pm 3.7\%$; $n = 4$) when the slices were perfused simultaneously with NMDA 4 μM (Fig. 11A).

The adenosine A_{2A} receptor agonist CGS21680 was perfused at a concentration of 30 nM since this has been shown previously to activate adenosine A_{2A} receptors selectively (O'Kane and Stone, 1998). At higher concentrations, it is also able to activate adenosine A_1 receptors. At 30 nM, CGS21680 did not itself modify the size of the population spikes but, when superfused together with NMDA, the population spike size was increased significantly by $16.5 \pm 1.3\%$ ($^{*}P < 0.05$; $n = 4$) compared with CGS21680 alone (Fig. 11B). The inclusion of the adenosine A_{2A} receptor-selective antagonist ZM241385 at 100 nM, prevented the excitatory effect of the combination of NMDA and CGS21680.

This interaction was still observed when tested in the paired-pulse paradigm. There was a clear trend for the combination of NMDA and CGS21680 to increase transmitter release, with a tendency for the paired-pulse interaction curve to be depressed at all tested time intervals (Fig. 12). However, this was only statistically significant at the 10-ms interstimulus interval. This significant difference was lost, however, in the presence of the antagonist

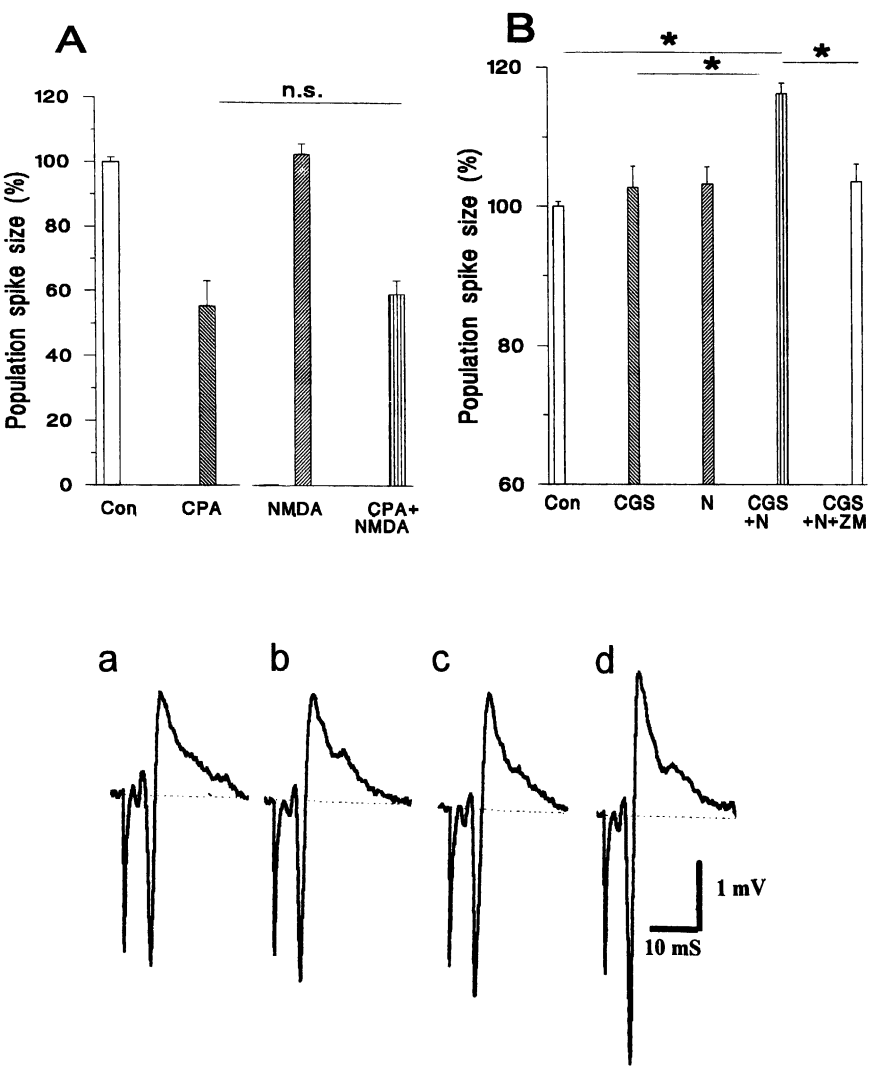

Fig. 11. Histograms summarizing the interactions between adenosine receptor ligands and NMDA. (A) Summarises the amplitude of control spikes expressed as 100% (Con), the inhibitory effect of CPA, 20 nM (CPA), the lack of effect of NMDA 4 μM alone (NMDA) and the effect of CPA in the presence of NMDA. (B) Summarises the amplitude of control spikes expressed as 100% (Con), the lack of response to CGS21680 30 nM alone (CGS) or NMDA alone (N), and the significant increase of potential size seen with the combination of CGS21680 and NMDA (CGS + N). This increase of potential size was prevented by the inclusion of ZM241385 (100 nM) in the solution (CGS + N + ZM). The columns indicate the mean ± S.E.M. ($n = 4$). Statistically significant differences between columns are indicated as $^{*}P < 0.05$, $^{*}P < 0.01$. The experimental records illustrate this interaction. (a) is a control population spike, (b) is in the presence of CGS21680 at 30 nM, (c) is in the presence of NMDA at 4 μM, and (d) is in the presence of CGS21680 and NMDA, when a significant increase of potential size was observed. Calibrations: 1 mV and 10 ms.

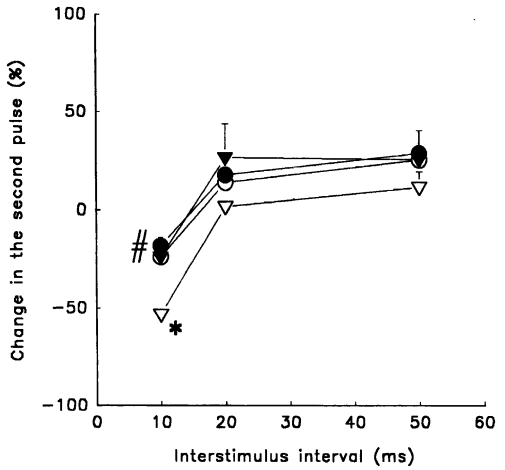

Fig. 12. The effect of CGS21680 on paired-pulse interactions. Data are shown from control slices (open circles), the lack of effect of CGS21680 (30 nM; closed circles), the increased inhibition produced by CGS21680 plus NMDA (4 μM) (open triangles), and the blockade of this increase by ZM241385 (100 nM) (closed triangles). Each point shows the mean ± S.E.M. ($n = 5$), with significant differences indicated between controls and CGS21680 plus NMDA ($^{*}P < 0.05$). The inclusion of ZM241385 then blocks this effect and restores paired-pulse inhibition to control values ($^{\#}P < 0.05$).

ZM241385, when the paired-pulse inhibition was restored to the control value, confirming the involvement of A_{2A} receptors in this change.

4. Discussion

In accord with many previous studies, adenosine at 10 μM depressed the population spike amplitude. This effect was prevented by superfusing the slices with NMDA at 4 μM, confirming the results of our original study (Bartrup and Stone, 1990).

The original studies on adenosine sensitivity and magnesium removal (Bartrup and Stone, 1988) were performed using population spike potentials, changes of which are normally considered to involve changes of postsynaptic excitability. A subsequent study of single cell responses to the activation of P1 adenosine receptors showed a depression of firing which was less apparent in the presence of NMDA than in the presence of quisqualic acid or acetylcholine (Bartrup et al., 1991). Although interpreted as consistent with a postsynaptic locus for the interaction between NMDA and adenosine receptors, it is difficult to be certain of the site of action of agents applied by microiontophoresis (Stone, 1985), and attempts to do so by, for example, lowering extracellular Ca^{2+}, complicate interpretation by modifying neuronal excitability and receptor function.

An alternative approach pursued in this study is to focus on presynaptic sites by the examination of paired-pulse interactions. Paired-pulse inhibition at interpulse intervals of around 10 ms reflects the depletion of transmitter from presynaptic stores (Burke and Hablitz, 1994; Wilcox and Dichter, 1994; Hashimoto and Kano, 1998), and is reduced by agents or procedures which decrease transmitter release. Paired-pulse facilitation, on the other hand, at longer interpulse intervals, results from the residual intraterminal Ca^{2+} which increases transmitter release (Hess et al., 1987; Wu and Saggau, 1994; Debanne et al., 1996; Kleschevnikov et al., 1997). There is already ample evidence for the existence of presynaptic glutamate receptors (Forsythe and Clements, 1990) and especially presynaptic NMDA receptors (Fink et al., 1990; Martin et al., 1991; Overton and Clark, 1991; Cai et al., 1991; Kato and Zorumski, 1999) on terminals in the hippocampus and other regions of CNS.

The present data show that the presynaptic adenosine and NMDA receptor populations can interact in such a way that NMDA receptor activation suppresses the inhibitory effects of adenosine on transmitter release assessed using paired-pulse interactions both with population spikes and population EPSPs. This interaction occurs at levels of NMDA receptor activation which are not themselves sufficient to alter paired-pulse inhibition and strongly suggests that the primary site of the interaction is presynaptic. The fact that the interaction can also be observed in the presence of bicuculline suggests that the receptors involved are likely to be located on the main terminals of the Schaffer collateral fibres, and not on inhibitory interneurones.

In addition, the suppression of adenosine sensitivity can be produced by methods other than the direct activation of NMDA receptors. Thus, the induction of long-term potentiation, which is known to involve the activation of NMDA receptors by synaptically released glutamate, also reduced adenosine responses.

As an alternative method of activating NMDA receptors the effects of glycine were also examined. Although the normal endogenous levels of glycine are believed to be adequate to saturate the strychnine-resistant binding site on the NMDA receptor, the application of exogenous glycine can enhance the activation of NMDA receptors (Minota et al., 1989) and this may be sufficient to induce or facilitate long-term potentiation in regions such as the hippocampus (Shahi and Baudry, 1993) and superior colliculus (Platt et al., 1998; Abe et al., 1990; Watanabe et al., 1992). Glycine-induced long-term potentiation in organotypic cultures is also due to activation of NMDA receptors, as it can be prevented selectively by NMDA antagonists (Newell et al., 1997; Tauck and Ashbeck, 1990). Although some of these effects may be triggered by the activation of NMDA receptors, such changes may be expressed by mechanisms, which involve associated changes of AMPA receptor function (Shahi and Baudry, 1993). The ability of 1 and 5 mM glycine in the present study to induce long-term potentiation which was prevented by 20 μM

dichlorokynurenate confirms the involvement of the glycine site of the NMDA receptor; since dichlorokynurenate has high selectivity for this site (Stone, 2000). The mechanistic similarity between electrically and glycine-induced long-term potentiation was further suggested by the occlusion of electrically induced long-term potentiation at a population spike size generated by saturating the long-term potentiation process.

The specificity of the interaction between NMDA or long-term potentiation and adenosine is indicated by the lack of any interaction with the $GABA_B$ receptor agonist baclofen which, like adenosine, is known to suppress transmitter release at low concentrations.

One explanation for some of the earlier data of Bartrup and Stone (1990) was proposed by Smith and Dunwiddie (1993), who argued that the effects of magnesium removal could simply reflect the altered balance between Ca^{2+} and magnesium in determining the amount of transmitter release and thus account for the loss of sensitivity to adenosine. However, the finding that application of NMDA itself mimicked the effects of low magnesium, while NMDA antagonists prevented it, indicates that this cannot represent the whole explanation and that amino acid receptors probably contribute to the phenomenon. Of course, it is still possible that the activation of NMDA receptors changes sensitivity to adenosine by way of an alteration of intracellular Ca^{2+} levels or availability to the transduction mechanism.

One of the most surprising observations of this study arose from an examination of adenosine receptor selective agonists, with the finding that, contrary to expectation, NMDA did not modify the inhibitory effects of cyclopentyladenosine, excluding an interaction between NMDA receptors and adenosine A_1 receptors as an explanation of the NMDA-induced loss of sensitivity to adenosine. Rather, the combination of a subthreshold concentration (4 μM) of NMDA and CGS21680 at a concentration known to be selective for the activation of adenosine A_{2A} receptors and which alone had no effect on the slices, together produced a significant increase of spike size.

The differential behaviour of NMDA towards adenosine A_1 and A_2 receptor activation implies that the apparent loss of sensitivity to adenosine produced by the presence of NMDA is not due to an antagonism of those adenosine actions responsible for decreasing transmitter release, but reflects a masking of the A_1-mediated inhibition by an enhancement of A_{2A} receptor function. Activation of the adenosine A_1 receptor subtype leads to the suppression of transmitter release, especially of glutamate, acetylcholine, norepinephrine and 5-hydroxytryptamine (Corradetti et al., 1984; Spignoli et al., 1984; Jonzon and Fredholm, 1984; Feuerstein et al., 1985; Prince and Stevens, 1992), probably by increasing potassium conductances or inhibiting Ca^{2+} movements or availability (Haas and Greene, 1984; Trussel and Jackson, 1985). The adenosine A_{2A} receptor population has been reported to enhance transmitter release (Correia-de-Sa et al., 1991; Sebastiao and Ribeiro, 1992; Kirkpatrick and Richardson, 1993; Latini et al., 1996; Cunha et al., 1997).

It is tempting to question whether the apparently small (but significant) increase of potential size which develops in the simultaneous presence of CGS21680 and NMDA is sufficient to account for the much more dramatic loss of adenosine sensitivity reported in this and earlier papers. At present, it is difficult to resolve this question, but it may be that other interactions of adenosine receptors are relevant. For example, it is now clear that the activation of adenosine A_{2A} receptors is able to suppress the effects of A_1 receptor activation (O'Kane and Stone, 1998; Latini et al., 1999; Cunha et al., 1994). The interaction between CGS21680 and NMDA is, therefore, likely to lead to an indirect decrease of adenosine A_1 receptor effects as well as producing a direct increase of excitability, and resulting in a further decrease of adenosine sensitivity.

This result appears particularly surprising in view of demonstrations that the activation of A_1 receptors in hippocampal neurones (De Mendonca et al., 1995) and A_{2A} receptors on striatal cells (Norenberg et al., 1997; Wirkner et al., 2000) can suppress neuronal responses to NMDA in slices and patch–clamp experiments. It should be emphasised, however, that the interactions described in the present study involved a concentration of NMDA which was not active when tested alone. It therefore seems that the simultaneous activation of adenosine A_{2A} and NMDA receptors at low (subthreshold) concentrations produce an increase of glutamate release and neuronal excitability, whereas their combined activation at higher concentrations —which are themselves depolarising results in antagonism.

Overall, therefore, NMDA receptor activation seems able to modify selectively the presynaptic responses to activation of A_{2A} adenosine receptors, leading to the masking of adenosine's inhibitory activity on transmitter release. The physiological significance of this is potentially interesting. Craig and White (1992) have proposed that adenosine A_1 receptors present a barrier to the actions of NMDA receptors which must be overcome if the full effects of NMDA receptor activation are to be observed in phenomena such as long-term potentiation. The present work suggests that part of the mechanism of overcoming this barrier may be that, under conditions in which the amount of adenosine released by neurons and glia is greatly increased so that the relatively low affinity A_{2A} receptors are activated, the inhibitory adenosine A_1 receptors effects are overcome. Such a sequence provides at least one rationale for the otherwise curious co-existence of inhibitory adenosine A_1 and facilitatory adenosine A_{2A} receptors on the same population of glutamatergic terminals, and is consistent with earlier proposals that A_{2A} receptor activation can suppress responses mediated by A_1 receptors (O'Kane and Stone, 1998; Latini et al., 1999; Cunha et al., 1994).

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