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Interaction between adenosine A_1 and A_2 receptor-mediated responses in the rat hippocampus in vitro

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Abstract

Previous work has been carried out on the effects of adenosine on transmitter release and on the excitability of postsynaptic neurones, but little is known about the effects of adenosine on the coupling between the two. In this study, we examine the effects of specific adenosine receptor agonists and antagonists on the population excitatory postsynaptic potential (population EPSP) slope, the population spike amplitude, and the relationship between the two (*E–S* coupling) in the CA1 area of rat hippocampus. Activation of adenosine A_1 receptors by adenosine or the selective agonist N^6-cyclopentyladenosine resulted in a decrease of the population spike amplitude by a greater extent than could be accounted for by the decrease in population EPSP slope, resulting in a dissociation in the *E–S* relationship, reflected as a right-shift in the *E–S* curve. Activation of adenosine A_{2A} receptors by the selective agonist 2-*p*-(2-carboxyethy)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680), or blockade by antagonists ZM 241385 and CP 66713 had no effect on evoked responses. However, when both adenosine A_1 and A_{2A} receptors were activated at the same time, a significant attenuation of the inhibitory effects of N^6-cyclopentyladenosine on population spike amplitude was observed, resulting in a left-shift in the *E–S* curve. Intracellular recording indicated that N^6-cyclopentyladenosine raised the threshold for spike induction by pulses of depolarising current, even at a concentration which did not produce hyperpolarisation of the neurone. At 30 nM, CGS 21680 prevented this effect of N^6-cyclopentyladenosine, and this apparent antagonism was prevented by the A_{2A} receptor antagonist ZM 241385. The results show that adenosine A_1 receptors change the coupling between presynaptic transmitter release and postsynaptic cell firing, and that this effect is attenuated by A_{2A} receptor activation.

Keywords: Adenosine receptor; Hippocampus; EPSP (excitatory postsynaptic potential)–spike relationship; Purine; Population EPSP; Population spike

1. Introduction

Adenosine is a neuromodulator with both presynaptic and postsynaptic effects in the mammalian central nervous system (CNS). It has been suggested that these effects are mediated by different mechanisms in view of, for example, their different sensitivities to agents such as pertussis toxin (Fredholm et al., 1989; Hasuo et al., 1992; Thompson et al., 1992). Presynaptically, adenosine has been shown to inhibit the release of neurotransmitters such as acetylcholine (Spignoli et al., 1984; Cunha et al., 1994b), dopamine (Michaelis et al., 1979; Zetterstrom and Fillenz, 1990), serotonin (Feuerstein et al., 1985), and glutamate (Fastbom and Fredholm, 1985). The mechanism of adenosine's presynaptic effects is not fully clear but it has been suggested that it causes an increase in potassium conductance which could hyperpolarise the axon terminal and so prevent transmitter release (Thompson et al., 1992). Supporting this, it has been shown that K^+ channel blockers such as 4-aminopyridine can reduce the effects of adenosine (Stone, 1981). An indirect decrease of Ca^{2+} currents or intracellular Ca^{2+} levels by this adenosine activated potassium conductance has also been suggested as a mechanism of presynaptic inhibition (Dunwiddie and Haas, 1985; Michaelis et al., 1988). Alternatively, adenosine may

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exert its presynaptic effects by inhibiting Ca^{2+} influx directly (Dolphin et al., 1986; Schubert et al., 1987).

Postsynaptically, the mechanism of action of adenosine is clearer. Several groups have suggested that adenosine causes an increase in outward potassium conductance leading to a hyperpolarisation of the postsynaptic membrane (Okada and Ozawa, 1980; Segal, 1982; Trussel and Jackson, 1985). This potassium conductance has been shown to be both Ca^{2+} and voltage insensitive in CA1 neurones (Gerber et al., 1989).

Earlier studies have suggested that the inhibitory presynaptic actions and postsynaptic hyperpolarising actions of adenosine are mediated by A_1 receptors (Dunwiddie and Fredholm, 1989; Alzheimer et al., 1991; Ameri and Jurna, 1991; Lambert and Teyler, 1991; Hasuo et al., 1992) which are present in high density in the hippocampus, especially in the CA1 region (Fastbom et al., 1987). Electrophysiological (Sebastião and Ribeiro, 1992) and binding (Jarvis et al., 1989; Cunha et al., 1994a) studies have shown that adenosine A_{2A} receptors are also present in the hippocampus but conflicting reports exist as to the functional importance of these receptors and their ability to interact with adenosine A_1 receptors. Cunha et al. (1994a) describe an attenuation of the inhibitory effects on population spike amplitude caused by activation of A_1 adenosine receptors with N^6-cyclopentyladenosine in the presence of the selective A_{2A} adenosine receptor agonist 2-*p*-(2-carboxyethy) phenethylamino - 5′ - *N*- ethylcarboxamidoadenosine (CGS 21680) in the rat hippocampus. In contrast, Dunwiddie et al. (1997) report that pre-treatment of hippocampal slices with CGS 21680 had no effect on subsequent responses to adenosine. Recently, Dixon et al. (1997) have shown the appearance of a low-affinity binding site for the adenosine A_1 receptor agonist 2-chloro-*N*-6-cyclopentyladenosine in the rat striatum caused by incubation of striatal synaptosomes with CGS 21680.

Although the aforementioned pre- and postsynaptic effects of adenosine have been well-characterised, little work has been carried out with regard to the effects of adenosine on the coupling between the two. This study investigates the effects of adenosine receptor agonists and antagonists on the population excitatory postsynaptic potential, the population spike, and the relationship between them i.e., EPSP–spike (*E–S*) coupling. The population excitatory postsynaptic potential (population EPSP) gives primarily a measure of membrane potential changes generated by excitatory synapses on the apical dendrites of CA1 pyramidal neurones. This extracellularly recorded potential has been shown to be affected by adenosine in the same way as intracellularly recorded EPSPs from the same cells (Proctor and Dunwiddie, 1987). The population spike reflects the summated firing of CA1 pyramidal neurones (Andersen et al., 1971) and gives a measure of the excitability of the postsynaptic neurone. *E–S* coupling gives an indication of the ability of a given level of synaptic depolarisation to induce the postsynaptic cell to fire an action potential. The three parameters measured can vary independently, and a description of all three is necessary to provide a full analysis of agents on neuronal function.

2. Method

Male Wistar rats (150–250 g) were anaesthetised with urethane (1.5 g/kg) i.p. and cooled on ice while breathing oxygen-enriched air until rectal temperature reached 30°C. This procedure was recommended by Newman et al. (1992) to enhance the viability of slices. The animals were then killed by cervical dislocation, decapitated, and the brain rapidly removed to ice-cold artificial cerebrospinal fluid (aCSF) of composition (mmol/l) KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10 saturated with 95% O_2 and 5% CO_2. The hippocampi were dissected free of surrounding tissue and were cut transversely into slices 450 μm thick using a McIlwain tissue chopper. Slices were maintained in an incubation chamber saturated with 95% O_2/5% CO_2 for at least 1 h before being transferred to a recording chamber and superfused with pre-gassed aCSF (34–35°C) at a constant flow rate of 2–3 ml/min. Drugs were added to the superfusion fluid

Test pulses (100 μs, 200–450 μA) were delivered at 20 s intervals via a concentric bipolar electrode placed in the stratum radiatum of the CA1 area. The preparation was allowed to stabilise for at least 45 min before recordings of extracellular population EPSPs and population spikes were made from the stratum radiatum and stratum pyramidale, respectively using glass microelectrodes of tip diameter approximately 2 μm and filled with 0.9% NaCl. Responses were quantified as the amplitude of the population spike, (measured as the difference between the peak negativity and the averaged values of the two peak positivities of the population spike), and the slope of the negative-going arm of the evoked population EPSP response. Five stimuli were delivered at each stimulation intensity and the average computed using a 32 channel signal averager (Cambridge Electronic Design). Input/output (*I/O*) curves were obtained by varying the stimulus intensity and plotting the responses for both population spikes and population EPSPs as a percentage of maximum control values against stimulation intensity. From these *I/O* curves, *E–S* curves were constructed by expressing population EPSP slopes and population spike amplitudes on the abscissae and ordinate, respectively. Changes in population EPSP slope and population spike amplitude were evaluated as differences in area under the curve of the respective *I/O* curves before and after drug addition for individual slices and the mean and S.E.M. calculated. As seen in previous investigations (Bernard and Wheal, 1995a,b) it proved difficult to pool the *E/S* curves due to the variability in the input/output properties of populations of neurones. Therefore, to quantify changes in the *E–S* relationship, the population spike

amplitude seen at a population EPSP slope of 70% of maximum control was measured before and after drug addition for each slice and values expressed as mean ± S.E.M. (Bernard and Wheal, 1995a,b).

Intracellular recordings were made using sharp microelectrodes filled with 1 M KCl or potassium acetate (90–120 MΩ). Potentials were amplified using a Neurolog NL 102 amplifier or Axoclamp-2A operated in bridge balance mode. Neurones were used if they displayed a stable resting potential greater than 60 mV and a spike of at least 70 mV amplitude. Depolarising current pulses were used to determine the threshold for spike initiation.

2.1. Drugs

Adenosine and N^6-cyclopentyladenosine were obtained from Sigma. 2-*p*-(2-carboxyethy)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were obtained from Research Biochemicals International. 4-Amino-8-chloro-1-phenyl(1,2,4)-triazolo(4,3a)quinoxaline (CP 66713) was obtained from Pfizer and 4-(2-[7-amino-2-{2-furyl}{1,2,4,}triazolo{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl)phenol (ZM 241385) from Zeneca Pharmaceuticals.

3. Results

3.1. Adenosine

The relationship between stimulation strength and population EPSP slope, population spike amplitude, and the corresponding *E–S* curve are summarised for a typical slice in Fig. 1. The addition of adenosine at 20 μM in this

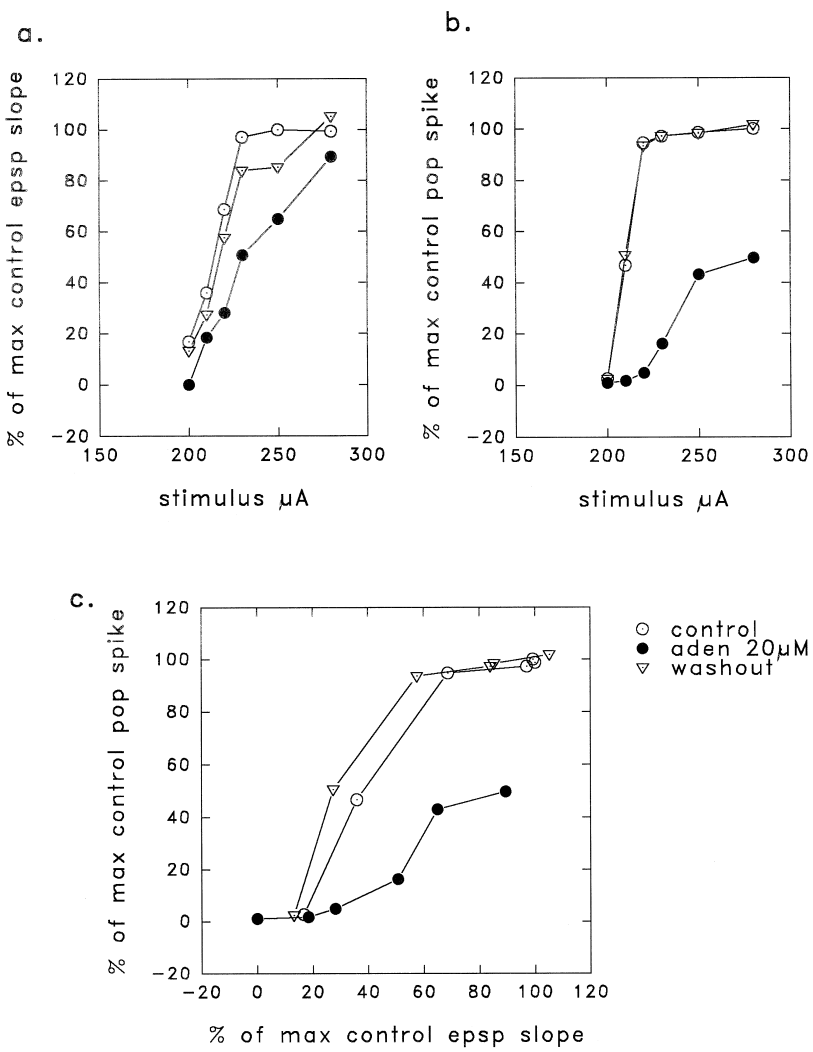

Fig. 1. Representative example of the input–output (*I/O*) curves and corresponding EPSP–spike (*E–S*) curve obtained upon addition of adenosine 20 μM. In all three curves, open symbols represent control conditions while filled symbols represent values on addition of adenosine. Curves (a) and (b) show *I/O* curves for population EPSP slope and population spike, respectively for the same slice. It can be seen that population spike is reduced by a greater extent than population EPSP. (c) Shows the *E–S* curve plotted from data from (a) and (b) showing a marked depression obtained upon addition of 20 μM adenosine.

case resulted in a significant decrease in both the population EPSP slope and population spike amplitude. From the population of slices examined, adenosine at 50 μM decreased both the population EPSP slope and population spike amplitude significantly ($n = 5$, $p < 0.01$ and $p < 0.001$, respectively). At the lower concentration of 20 μM, adenosine also decreased these potentials to a significant degree ($n = 5$, $p < 0.05$ and $p < 0.01$, respectively) although to a smaller extent. (Fig. 2). The population spike amplitude was reduced by a greater extent than can be accounted for by the decrease in population EPSP slope, resulting in a shift to the right of the *E–S* curve of 24.7% ± 10.2 ($p < 0.05$) with adenosine 20 μM and 43.5% ± 15.1 with adenosine 50 μM ($p < 0.05$) (Fig. 2).

3.2. Selective agonists and antagonists

The selective adenosine A_1 receptor agonist N^6-cyclopentyladenosine 50 nM also caused a significant decrease in the population EPSP slope and population spike amplitude (28.9 ± 7.9%, $n = 5$, $p < 0.01$ and 83.9 ± 5.2%, $n = 5$, $p < 0.001$, respectively, Fig. 2c). This resulted in a large shift to the right of the *E–S* curve of 81.8 ± 5.2% ($p < 0.001$) (Fig. 2d). To ensure that N^6-cyclopentyladenosine was exerting its effects on population EPSP slope as a result of decreased transmitter release and not due to influencing presynaptic afferent excitability, the slope of the presynaptic volley was also measured in three experiments and was plotted as a function of stimulus intensity in the same manner as *I/O* curves for population spike amplitude and population EPSP slope. No change was observed in presynaptic afferent excitability with N^6-cyclopentyladenosine, showing that its effects are restricted to events occurring subsequent to the arrival of the fibre volley at the striatum radiatum axon terminals.

The selective A_{2A} agonist 2-*p*-(2-carboxyethy)-phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) 30 nM ($n = 5$) and 100 nM ($n = 4$) had no signifi-

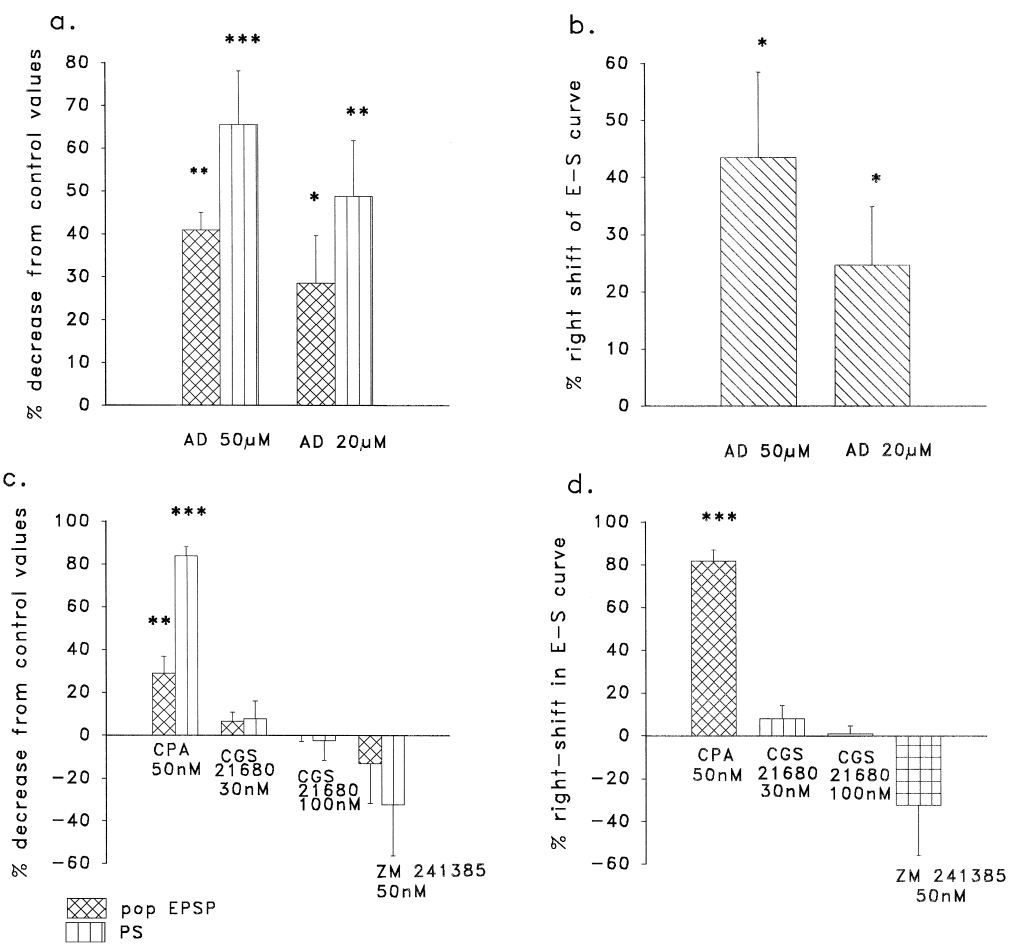

Fig. 2. Pooled data from five slices showing (a) percentage decrease in population EPSP slope and population spike amplitude and (b) percentage shift in *E–S* curves from control values upon addition of adenosine 20 μM and 50 μM. Histograms in (c) and (d) show the effects of the addition of adenosine agonists and antagonists on (c) input/output curves and (d) the corresponding *E–S* curves. The A_1 receptor agonist N^6-cyclopentyladenosine decreases both population EPSP slopes and population spike amplitudes, and shifts the *E–S* curve to the right, whereas A_{2A} receptor activation has no significant effects on any of the three parameters. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with controls.

cant effects on population EPSP slope, population spike amplitude or the relationship between the two. Similarly, the A_{2A} antagonists 4-(2-[7-amino-2-{2-furyl}{1,2,4,}-triazolo{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl)phenol (ZM 241385 50 nM) and 4-amino-8-chloro-1-phenyl(1,2,4)-triazolo(4,3a)quinoxaline (CP 66713 10 μM) also showed no significant effects on the three parameters, although ZM 241385 showed a tendency to increase all three (Fig. 2c and d).

3.3. $A_1 \backslash A_2$ interactions

As previously mentioned, different reports have emerged on the ability of adenosine A_1 and A_2 receptors to interact with each other (Cunha et al., 1994a; Dunwiddie et al., 1997). When a combination of N^6-cyclopentyladenosine and CGS 21680 was added to the slices, there was a reduction in the effects of N^6-cyclopentyladenosine on population EPSP slope and population spike amplitude, with CGS 21680 30 nM causing a significant attenuation in the reduction of population spike amplitude seen with N^6-cyclopentyladenosine alone ($p < 0.05$) (Fig. 3a). CGS 21680 at both 30 nM and 100 nM caused a concentration-related but significant reduction in the effect of N^6-cyclopentyladenosine on $E–S$ coupling as summarised in Fig. 3b.

3.4. Intracellular recording

At concentrations of 50 or 100 nM, N^6-cyclopentyladenosine produced very little or no change of resting membrane potential (mean hyperpolarisation of 1.8 ± 1.2 mV at 100 nM, $n = 10$). Nevertheless, N^6-cyclopentyladenosine raised the threshold for spike initiation in 11 of 12 neurones in which this was examined. In all of four of these cells tested, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 nM) prevented this effect of N^6-cyclopentyladenosine. Fig. 4A illustrates a continuous recording of membrane potential in which a depolarising current pulse was applied every 20 s. The size of the current pulse was adjusted to a level which was just sufficient to evoke an action potential on each occasion. The addition of N^6-cyclopentyladenosine at 100 nM caused no hyperpolarisation in the cell of Fig. 4A, but raised the spike threshold such that the cell failed to trigger an action potential on most occasions. When CGS 21680 at a concentration of 30 nM was perfused for 15 min before and together with

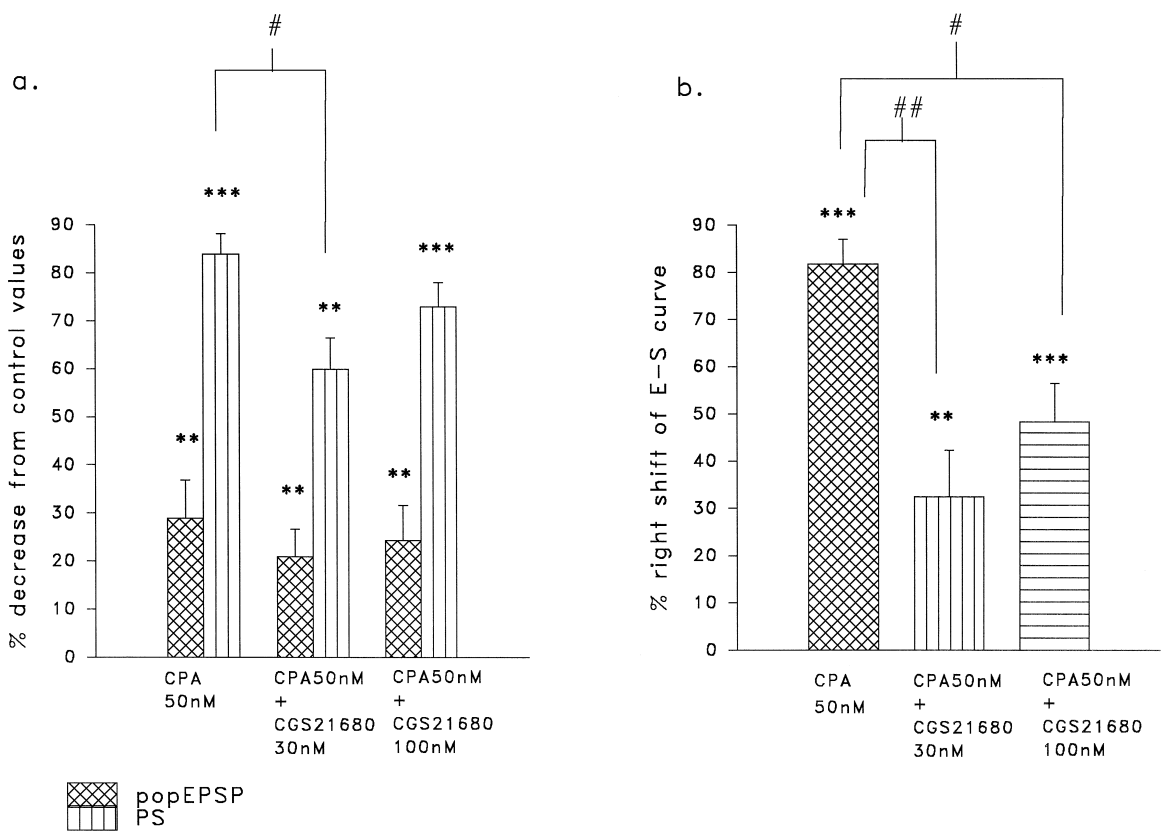

Fig. 3. Pooled data showing the effects of co-activation of A_1 and A_{2A} receptors with selective agonists. Addition of the A_{2A} receptor agonist CGS 21680 causes a significant reduction in the population spike amplitude seen upon A_1 activation alone (a). Addition of either 30 nM or 100 nM CGS 21680 results in a significant reduction in the right-shift of the $E–S$ curve seen upon A_1 receptor activation (b). ** $p < 0.01$; *** $p < 0.001$ compared with controls. # $p < 0.05$; ## $p < 0.01$ between columns.

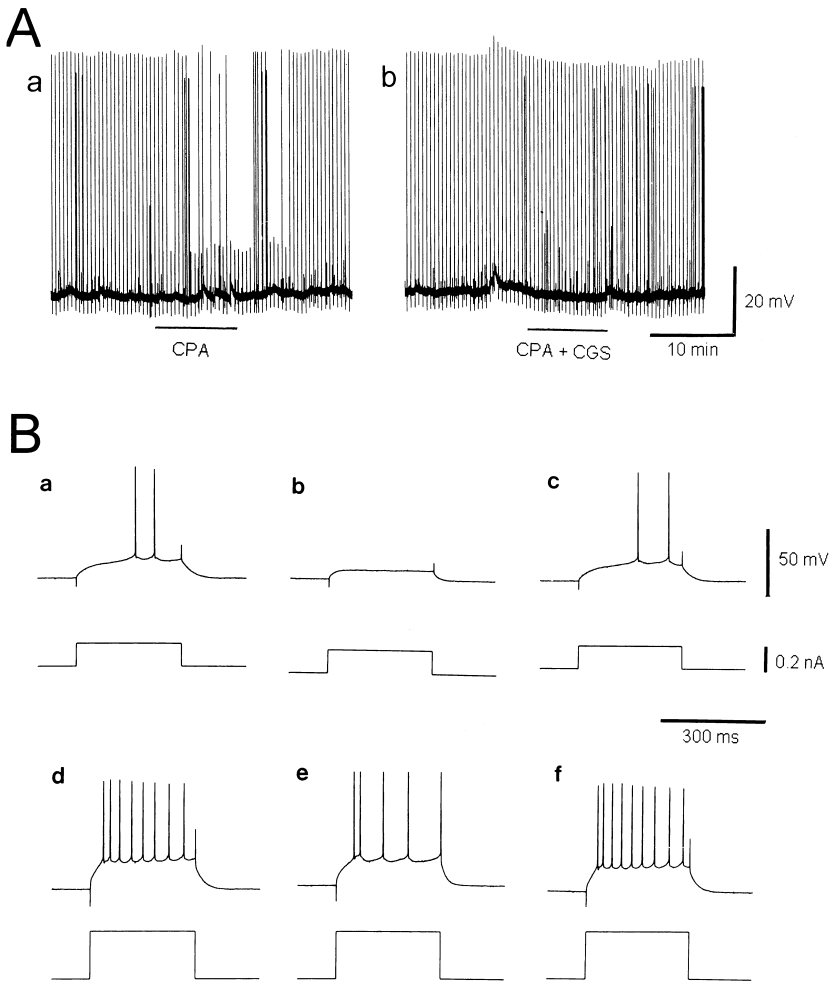

Fig. 4. Intracellular recordings from pyramidal neurones in the hippocampus. Record A(a) illustrates the membrane potential of a neurone which is stimulated by a depolarising current pulse just sufficient to induce the production of an action potential on most occasions (0.22 nA delivered every 30 s). During the period indicated by the bar below the record, N^6-cyclopentyladenosine was perfused at 100 nM, and causes failure of action potential initiation with no accompanying change of membrane potential. In record A(b), the cell is superfused with N^6-cyclopentyladenosine 100 nM plus CGS 21680 30 nM. The latter compound was perfused for 15 min before the addition of CPA. The elevation of spike threshold is now blocked, and there is some evidence of increased synaptic activity and spontaneous action potentials, with little change of overall membrane potential. Records B show this effect on a more expanded time scale from a different cell. B(a) and (d) represent responses of the cell to pulses of 0.2 and 0.4 nA in the control state. Record (b) shows the failure of spike initiation and (e) a reduced number of spikes produced during superfusion with N^6-cyclopentyladenosine 100 nM. The latter record (e) also shows an increase in the degree of after-hyperpolarisation which probably contributed to the reduced spike number. Records (c) and (f) show the prevention of the N^6-cyclopentyladenosine effect when co-perfused with CGS 21680 30 nM. Calibrations: 20 mV and 10 min in A; 50 mV, 0.2 nA and 300 ms for B.

N^6-cyclopentyladenosine, the change of spike threshold was prevented (Fig. 4A). Examples of this same effect are shown in more detail in Fig. 4B, which illustrates the responses of a neurone to intracellularly applied current pulses. The suppressant effect of N^6-cyclopentyladenosine is reflected both in the blockade of spike initiation, and as a reduction in the number of evoked spikes at higher current intensities.

When the A_{2A} receptor antagonist ZM 241385 was perfused over the slices at a concentration of 100 nM for 15 min before the addition of CPA with CGS 21680, the inhibitory effect of the CPA was restored. This effect was noted in all of six neurones tested, ZM 241385 being able to prevent completely the effect of CGS 21680 in blocking CPA on four neurones, while only a partial blockade was observed in the other two cells.

4. Discussion

The results reveal that adenosine reduces both the population EPSP slope and population spike amplitude in the CA1 area of rat hippocampus, showing that it has both pre- and postsynaptic actions. Consistent with previous findings (Dunwiddie and Fredholm, 1989; Alzheimer et al., 1991; Lambert and Teyler, 1991), these effects are mediated by adenosine A_1 receptors as shown by the fact that the

selective A_1 agonist N^6-cyclopentyladenosine produced a similar depression of both potentials while the A_{2A} agonist CGS 21680 had no significant effects. N^6-cyclopentyladenosine caused a significantly greater shift in the *E–S* curve than adenosine, reflecting the greater effect of N^6-cyclopentyladenosine on population spike amplitude combined with a lesser reduction in population EPSP slope compared with adenosine. This could possibly be due to a greater number of adenosine A_1 receptors at postsynaptic sites (Deckert and Jorgensen, 1988) or a greater proportion of A_{2A} receptors at postsynaptic sites which, when activated by adenosine cause an attenuation of the effects of A_1 receptor activation. Alternatively, this difference could be explained by a more efficient uptake system for adenosine operating at presynaptic compared with postsynaptic sites. The result is consistent with an earlier report that the A_1 receptor antagonist DPCPX reduced the threshold for spike initiation (Schubert, 1988).

E–S dissociation has previously been described as a component of long-term potentiation in the CA1 region of rat hippocampus (Andersen et al., 1980; Bernard and Wheal, 1995a,b). Two mechanisms have been put forward to explain this *E–S* dissociation. The first is a change in inhibitory pathways (Chavez-Noriega et al., 1989; Tomasulo et al., 1991; Tomasulo and Ramirez, 1993) such as a modification of excitatory synapses on interneurones or changes in inhibitory synapses. Secondly, *E–S* dissociation may occur via a postsynaptic mechanism which alters the firing threshold of the cells (Taube and Schwartzkroin, 1988; Hess and Gustafsson, 1990). It should be noted that these two mechanisms are not mutually exclusive. It has been suggested that adenosine modulates excitatory but not inhibitory synaptic transmission in the hippocampus (Lambert and Teyler, 1991; Yoon and Rothman, 1991; Brundege and Dunwiddie, 1996) and a decrease in transmitter release here may account for some of its actions on the *E–S* relationship. However, since the population spike amplitude, which reflects postsynaptic excitability, decreased more than could be accounted for by the decrease in transmitter release, it would appear that postsynaptic mechanisms play the major role in the decrease in *E–S* coupling seen with adenosine A_1 receptor activation. This presumably results from the increased potassium conductance which increases the firing threshold of the cell (Segal, 1982; Trussel and Jackson, 1985; Thompson et al., 1992). This conclusion is strongly supported by the intracellular experiments, since N^6-cyclopentyladenosine proved able to inhibit spike initiation at a concentration of 100 nM which had little effect on resting potential.

The mechanism by which the change of *E–S* coupling occurs remains unclear. It is possible that there is a change in the postsynaptic threshold for spike initiation at the level of the spike-generating machinery, but it is also possible that the change involves an alteration of dendritic length constant. It is unlikely that a change of inhibitory transmitter tone is involved as there was no significant evidence of postsynaptic hyperpolarisation in the intracellular recordings.

Conflicting evidence exists as to the ability of adenosine A_{2A} receptors to interact with A_1 responses (Cunha et al., 1994a; Dixon et al., 1997; Dunwiddie et al., 1997). In this study, we have seen a clear attenuation of the population spike response obtained following activation of adenosine A_1 receptors in the presence of the A_{2A} agonist CGS 21680, showing there is a cross-talk between the two classes of receptor. Adenosine A_{2A} receptor activation had no significant effect on the reduction of population EPSP slopes seen with A_1 receptor activation suggesting that A_{2A} receptors interact with A_1 receptors selectively at postsynaptic sites. Previous reports suggest that adenosine A_{2A} receptors have an excitatory function in the cortex (O'Regan et al., 1992), striatum (Kirkpatrick and Richardson, 1993) and hippocampus (Sebastião and Ribeiro, 1992), although Cunha et al. (1994a) suggest that this excitatory function is only apparent in the CA3 region and dentate gyrus. If this were the case here, it would be expected that when both inhibitory A_1 and excitatory A_{2A} receptors are stimulated, the net synaptic activity would be the result of the summation of the inhibitory vs. excitatory mediated responses. However, this seems an unlikely explanation since we saw no significant excitatory response with the 30 nM A_{2A} receptor selective concentration of CGS 21680 used alone, but an attenuation of A_1 receptor-mediated inhibitory responses in its presence. The selectivity of this interaction is well-illustrated by the intracellular results, since CGS 21680 was able to prevent the suppression of spike initiation by N^6-cyclopentyladenosine, in the absence of any significant changes of resting potential.

The interaction between adenosine A_1 and A_2 receptors could be of biological importance in situations in which the levels of endogenous adenosine rise. The levels of adenosine in normal brain are around 1 μM (Dunwiddie et al., 1981; Fredholm et al., 1984; Newman and McIlwain, 1977), sufficient to explain the behavioural and electrophysiological effects of inhibition of A_1 receptors (Dunwiddie et al., 1981; Snyder et al., 1981). In conditions where the levels of adenosine in the brain rise, for example, in ischaemia, A_{2A} receptors may also be activated which would attenuate the inhibitory and neuroprotective effects of A_1 receptor activation (MacGregor et al., 1993).

The non-xanthine compound ZM 241385 has a selectivity for A_{2A} receptors relative to A_{2B} and A_1 of approximately 32 and 420-fold, respectively, with a pA_2 value in the low nanomolar range at A_{2A} sites (Poucher et al., 1995). In the hippocampus, a concentration of 20 nM has been reported as sufficient to fully block the stimulant activity of CGS 21680 (Cunha et al., 1997). The concentration of ZM 241385 used for the present experiments was 100 nM, which should, therefore, completely prevent activation of A_{2A} receptors. In four of six neurones tested, ZM 241385 was able to prevent completely the effect of CGS 21680 in blocking CPA, while only a partial block-

ade was observed in the other two cells. While this seems consistent with a role for A_{2A} receptors, it should be noted that CGS 21680 has been reported as binding to two sites in the rat hippocampus, the predominant one of which does not exhibit the 'classical' A_{2A} pharmacology of the striatal site (Cunha et al., 1996).

A possible explanation for the observed A_1/A_2 receptor interaction is that activation of the adenosine A_{2A} receptor binding site by CGS 21680 may lead to a conformational change in the A_1 receptor and result in a decrease in the affinity of the receptor binding site for the A_1 agonist. This process would not require a second messenger system. Autoradiographic studies show binding of [^{3}H]CGS 21680 to be greatest in the stratum radiatum, and adenosine A_1 and A_{2A} receptors have previously been found together presynaptically at rat cholinergic nerve terminals (Correia-De-Sá et al., 1991; Cunha et al., 1994b). The close proximity of the two receptors may allow for this conformational interaction to occur. Dixon et al. (1997) reported the appearance of a low affinity binding site for the A_1 agonist 2-chloro-*N*-6-cyclopentyladenosine following incubation of striatal synaptosomes with CGS 21680. This was interpreted as desensitisation by the A_{2A} site of the A_1 receptor. Such an explanation could account for the present observations although the interaction reported by Dixon et al. (1997) was mediated by protein kinase C.

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