Barium, Glibenclamide and CGS21680 Prevent Adenosine A₁ Receptor Changes of ES Coupling and Spike Threshold

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Key Words

Adenosine, ES coupling · Barium channels · EPSP spike coupling

Abstract

Activation of adenosine A₁ receptors raised spike thresholds and induced a dissociation of excitatory postsynaptic potential (EPSP) spike coupling in hippocampal pyramidal neurones. This effect could be prevented by activation of A_{2A} adenosine receptors. The A₁ receptor agonist N⁶-cyclopentyladenosine caused a dissociation of the EPSP spike coupling recorded extracellularly and increased the threshold for spike generation measured intracellularly. These effects were prevented by the A_{2A} receptor agonist CGS21680. A series of agents interfering with adenylate cyclase activity, protein kinase A or C, or nitric oxide synthase had no effect on these responses to N⁶-cyclopentyladenosine. Superfusion with barium or glibenclamide prevented both the dissociation of EPSP spike coupling and the increase of spike threshold. It is concluded that a barium- and glibenclamide-sensitive potassium current may be involved in the postsynaptic effects of A₁ receptors on spike threshold, and it is suggested that a similar suppression of a potassium current by A_{2A} receptors could underlie the inhibition of A₁ receptor responses.

Introduction

Adenosine is an important neuromodulator in both the central and peripheral nervous systems acting both pre- and postsynaptically [1]. To date, 4 types of adenosine receptor have been cloned, namely adenosine A₁, A_{2A}, A_{2B}, and A₃ receptors [2–5]. Several groups have previously reported an inhibitory effect of adenosine A₁ receptors on population field excitatory postsynaptic potentials (fEPSPs), population spikes (PS) and the relationship between the two, i.e. EPSP spike (ES) coupling in the CA1 area of rat hippocampus (see [6]). The fEPSP gives primarily a measure of membrane potential changes generated by excitatory synapses on the apical dendrites of CA1 pyramidal neurones. The population spike reflects the summated firing of CA1 pyramidal neurones [7] and gives a measure of the excitability of the postsynaptic neurone. EPSP spike coupling gives an indication of the ability of a given level of synaptic depolarisation to induce the postsynaptic cell to fire an action potential. Intracellularly, changes in excitability can be measured as a change in EPSP slope or as a change in the firing probability of the cell [8–11].

We have observed that activation of adenosine A_{2A} receptors can attenuate the adenosine A₁ receptor-mediated inhibition of EPSP spike coupling by decreasing the effects of adenosine A₁ receptor activation at postsynaptic sites [6]. In this study we attempt to determine the mechanism by which adenosine receptors dissociate

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EPSP spike coupling using modulators of second messenger systems, potassium channel blockers, and nitric oxide synthase inhibitors.

Methods

Male Wistar rats (150–250 g) were anaesthetised with urethane (1.5 g/kg) i.p. 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⁻¹) KH₂PO₄ 2.2, KCl 2, NaHCO₃ 25, NaCl 115, CaCl₂ 2.5, MgSO₄ 1.2, glucose 10 saturated with 95% O₂ and 5% CO₂. The hippocampi were dissected free of surrounding tissue and were cut transversely into 450-µm thick slices using a McIlwain tissue chopper. Slices were maintained in an incubation chamber saturated with 95% O₂/5% CO₂ 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 superfusing aCSF.

Test pulses (0.05 Hz) were delivered 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 field EPSPs and population spikes were made from the stratum radiatum and stratum pyramidale respectively, using glass microelectrodes with a tip diameter of approximately 2 µm and filled with 0.9% NaCl (2–5 Megohms resistance). Following the attainment of a stable maximum potential size the stimulus strength was reduced to yield a potential approximately 70% of that maximum in order to ensure that both increases and decreases of response size could be detected. Slices were only accepted for experimental use if the PS was more than 2 mV and the fEPSP more than 1 mV in amplitude, both potentials remaining stable (within 10% of their mean amplitude) for at least 20 min before any drug application.

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 fEPSP response. Five stimuli were delivered at each stimulation intensity and the average computed using a 32-channel signal averager (Cambridge Electronic Design Ltd). Input/output (I/O) curves were obtained by varying the stimulus intensity and plotting the responses for both population spikes and fEPSPs as a percentage of maximum control values against stimulation intensity (fig. 1). From these I/O curves E-S relationships were measured by expressing PS amplitudes as a function of fEPSP slopes in the ratio PS/fEPSP for each slice, the results were expressed as mean ± SE mean. Changes in fEPSP 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 SE mean calculated. The area was calculated as the integral between 0 and 100% values on the abscissa, using the graphics programme SigmaPlot. Changes in E-S relationship are expressed as percentage differences in the ratio PS/fEPSP after drug addition compared with the control ratio. In order to compare the shifts in E-S coupling obtained with different drug treatments, we have calculated the E-S curve shift. This shift was expressed as the change in PS amplitude measured at a single stimulus strength which induced an fEPSP at 70% of its maximum.

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.

Drugs

Adenosine, N⁶-cyclopentyladenosine (CPA), 8-bromoadenosine-3',5'-cyclic monophosphate (8Br-cAMP), N^G-nitro-*L*-arginine methyl ester (*L*-NAME), 7-nitroindazole (7NI), barium chloride and forskolin were obtained from Sigma Chemical Co. Ltd. 2-*p*-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS21680), and N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide hydrochloride (H8) were obtained from Research Biochemicals International. N-(2-aminoethyl)-5-chloro-1-naphthalenesulfonamide (A3 HCl), and 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl)maleimide (GF 109203X) were obtained from Tocris Cookson. ZM241385 (4-(2-[7-amino-2-(2-furyl)]1,2,4-triazolo[2,3-a][1,3,5]triazin-5-ylamino)ethyl)phenol was a generous gift of Dr. S. Poucher, AstraZeneca.

Results

The effect of a 10-min perfusion of CPA 50 nM was to decrease PS amplitude more than the fEPSP slope, resulting in an uncoupling of the E-S relationship (fig. 1). I/O data revealed a decrease in PS amplitude of $83.9 \pm 4.3\%$ and fEPSP slope of $28.9 \pm 7.9\%$, resulting in a reduction of the ratio PS/fEPSP from 0.79 ± 0.1 in controls to 0.15 ± 0.05 ($p < 0.001$, $n = 3$) following perfusion of CPA, a decrease of $80.1 \pm 6.4\%$. When 2-*p*-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS21680), 30 nM was perfused 10 min before and during the addition of CPA 50 nM, the effects of CPA on PS amplitude and fEPSP slope were inhibited, although this concentration of CGS21680 had no effect alone on either PS size or fEPSP slope. The inhibitory effect of CPA on PS amplitude was affected by CGS21680 to a greater extent than the effect on fEPSP slope, indicating that the effect of CGS21680 was being exerted primarily at the postsynaptic surface. This resulted in a smaller EPSP spike dissociation than that seen upon A₁ receptor activation alone. In this case the decrease in PS amplitude was $56.9 \pm 6.5\%$ and fEPSP slope was $20.9 \pm 5.7\%$, reflecting a significant attenuation of the E-S dissociation caused by adenosine A₁ receptor activation compared with CPA alone ($p < 0.05$, $n = 5$).

Effects of Barium and Glibenclamide

Superfusion of the potassium channel blocker, barium 500 µM to the slice 10 min prior to, and during, CPA

Fig. 1. I/O plot constructed from measurements of the amplitude of the population spike and the slope of the fEPSP under control conditions (■) and in the presence of CPA (□). Graph illustrates the greater depression of the fEPSP compared with the population spike by CPA. The E-S shift was quantified as the ratio of the areas under the two curves between 0 and 100% on the abscissa scale. Symbols indicate mean values from 6 slices. The traces on the left are representative records of the population spike potential (a, b) and field EPSP (c, d) in normal medium (a, c) and during the superfusion of CPA 50 nM (b, d). In this example the population spike size is reduced by 64% at a time when the simultaneously recorded fEPSP slope is only reduced by 16%.

Fig. 2. A One method of measuring intracellular changes of spike threshold was to use depolarising currents of increasing strength and count the number of action potentials elicited. The responses illustrated are of one cell to a stimulus of 0.06 nA (threshold in the absence of drugs) and then at 2, 3, 4 and 5 times that stimulus strength applied for the duration indicated in the final record (at $5 \times$ threshold). **B** This graph summarises the number of spikes produced in the absence of drugs (control), and in the presence of CPA, CPA + CGS21680, or CPA + glibenclamide (glib). Symbols indicate mean $\pm$ SE mean for $n = 5$. All points in the upper 3 curves are significantly different from CPA ($p < 0.05$ at $1 \times$ threshold; $p < 0.001$ at all other points).

50 nM perfusion, attenuated the inhibitory effects of CPA on PS amplitude, again resulting in a significant inhibition of the E-S dissociation seen with CPA alone. The PS/fEPSP ratio was changed from $80.1 \pm 6.4\%$ in the presence of CPA alone to $51.3 \pm 9.4\%$ with CPA and CGS21680, and to $45.2 \pm 8.3\%$ with CPA, CGS21680 and barium (both $p < 0.05$, $n = 5$). The changes in PS/fEPSP ratio seen when adenosine A_{2A} receptors were activated with CGS21680 ($20.3 \pm 4.5\%$, $n = 5$) or when potassium channels were blocked with barium ($16.8 \pm 5.7\%$, $n = 5$) were not significantly different.

Similarly glibenclamide 10 μM had no effect when applied alone to slices (change of PS/fEPSP ratio of $18.6 \pm 4.3\%$, $n = 5$), but significantly attenuated the effects of CPA on PS amplitude to a greater extent than the fEPSP, diminishing the CPA-induced E-S dissociation.

Intracellular Studies

Intracellular pulses of depolarising current were used to elicit action potentials. A representative series of responses are illustrated in figure 2A, together with a graph of the mean spikes evoked per response (mean of 5 con-

Fig. 3. Inset illustrates the use of a depolarising current ramp to determine the threshold for spike initiation. Main histogram summarises the elevation of threshold obtained by this method (normalised to the control = 1) by CPA, the blockade of the CPA effect by CGS21680, and the blockade of the CGS21680 effect by ZM241385. Each column shows mean $\pm$ SEM for $n = 5$. ^a $p < 0.001$ compared with control; ^b $p < 0.001$ compared with CPA + CGS21680.

secutive stimuli) plotted against stimulus strength (fig. 2B). The graph shows the depressant effect of CPA on the number of evoked spikes and the prevention of that effect by the simultaneous addition of CGS21680.

The effect of CPA on E-S coupling is confirmed by the intracellular demonstration of an increase in spike threshold seen in figure 3. This change of threshold was prevented by the simultaneous perfusion of CGS21680 (fig. 3), but was not accompanied by any change of spike profile (fig. 4A, B, 5) or by any change of membrane input resistance. Barium and glibenclamide had an exactly similar action, reducing the effect of CPA on spike threshold without any change of spike profile or input resistance. Neither CGS21680, barium, nor glibenclamide had any effect themselves on the spike threshold, making it unlikely that a simple physiological antagonism could account for their effects on reducing the actions of CPA.

Fig. 4. Intracellular recordings illustrating the effect of CPA 50 nM on spike thresholds. In these examples a depolarising current injection of 0.15 nA was used to elicit action potentials under control conditions (A), in the presence of CPA, which raises the action potential threshold (B) and in the combined presence of CPA and barium (C) or CPA and glibenclamide (D), both of which prevent the depressant effect of CPA.

Other Transduction Modulators

Several agents that modify signal transduction processes were tested, including the adenylate cyclase activator forskolin and modulators of protein kinases. While some of these compounds had effects of their own on the fEPSP or PS, none produced any change in the effects of CPA on these parameters or on EPSP spike coupling (table 1).

Discussion

The adenosine A₁ receptor-selective agonist CPA depressed the amplitudes of PS significantly more than fEPSP slopes. This suggests that the EPSP-spike dissociation is mainly caused by actions of CPA at postsynaptic sites [6]. These actions of CPA, however, are prevented by the presence of the A_{2A} receptor-selective agonist CGS21680,

Table 1. Effects of modulators of different transduction systems on population EPSP slopes (fEPSP), population spike (PS) amplitudes and E-S coupling used both alone and in combination with CPA 50 nM

Drug/Combination	n	fEPSP decrease, %	PS decrease, %	E-S right shift, %
CPA 50 nM	5	28.9 ± 7.9 ^b	83.9 ± 4.3 ^c	81.8 ± 5.2 ^c
8Br cAMP 100 μM	6	28.2 ± 8.04	56.7 ± 11.8 ^b	23.4 ± 20.2
8Br cAMP 100 μM + CPA 50 nM	6	46.7 ± 5.8 ^a	92.0 ± 4.4 ^b	66.9 ± 15.4 ^a
Forskolin 10 μM	6	55.8 ± 3.1 ^c	82.0 ± 2.4 ^c	59.4 ± 7.2 ^b
Forskolin 10 μM + CPA 50 nM	6	56.8 ± 2.9 ^{c,d}	91.5 ± 2.0 ^c	88.6 ± 3.1 ^c
SQ22536 10 μM	6	-9.0 ± 13.8	-5.3 ± 9.6	-10.4 ± 4.5
SQ22536 10 μM + CPA 50 nM	6	29.7 ± 7.4 ^b	81.3 ± 3.9 ^c	53.7 ± 19.5
A3 HCl 10 μM	4	3.96 ± 12.4	4.3 ± 12.8	-19.6 ± 12.4
A3 HCl 10 μM + CPA 50 nM	4	37.1 ± 8.0 ^a	86.9 ± 8.0 ^b	62.9 ± 19.1 ^a
GF109203X 100 nM	5	19.9 ± 14.2	35.2 ± 31.1 ^a	9.786 ± 9.7
GF109203X 100 nM + CPA 50 nM	5	32.1 ± 16.2 ^a	95.3 ± 4.1 ^c	78.9 ± 9.7 ^c
H8 30 μM	6	-9.3 ± 13.6	-30.8 ± 11.8	-25.3 ± 22.7
H8 30 μM + CPA 50 nM	6	22.17 ± 9.23	75.8 ± 8.3 ^b	63.9 ± 23.4 ^a
7-NI 40 μM	5	18.4 ± 8.0	15.9 ± 13.8	-2.0 ± 10.0
7-NI 40 μM + CPA 50 nM	5	41.7 ± 8.7 ^a	80.5 ± 14.1 ^c	73.3 ± 17.6 ^c
L-NAME 20 μM	4	17.5 ± 13.5	25.1 ± 24.7	2.5 ± 5.0
L-NAME 20 μM + CPA 50 nM	4	39.9 ± 9.5 ^a	91.7 ± 4.3 ^c	89.2 ± 5.4 ^c

Data for CPA 50 nM are included for comparison. Only the adenylate cyclase activator forskolin 10 μM and the protein kinase C inhibitor GF109203X 100 nM had significant effects on any of the three parameters when used alone. None of the agents, when superfused in combination with CPA 50 nM, showed any significant differences in population spike amplitude or E-S coupling relative to the responses seen when CPA was used alone. This was true also for population EPSP slopes, with the exception of forskolin, which had a large effect on population EPSP slopes by itself.

None of the agents used mimicked the attenuation of CPA inhibitory responses seen upon adenosine A_{2A} receptor activation with CGS 21680, suggesting they do not play a role in the mechanism by which adenosine A_{2A} receptor activation attenuates A₁-mediated responses.

^a Significantly different from control values ($p < 0.05$); ^b significantly different from control values ($p < 0.01$); ^c significantly different from control values ($p < 0.001$); ^d significantly different from CPA 50 nM ($p < 0.05$).

consistent with the existence of cross-talk between the two subtypes of receptor. (The attenuation of A₁ receptor-mediated responses by the activation of adenosine A_{2A} receptors is not due to a functional antagonism between excitatory versus inhibitory effects of the two receptor types, since the activation of A_{2A} receptors separately by CGS21680 does not cause any apparent excitation in terms of changes in EPSP or PS size [6].) An attenuation of adenosine A₁ receptor responses by A_{2A} receptor activation in the rat hippocampus has previously been reported by Cunha et al. [12], and a phenomenon which is probably related to this – desensitisation of adenosine A₁ receptors by A_{2A} receptors in the rat striatum – was described by Dixon et al. [13].

This latter interaction was mediated by protein kinase C, whereas in the hippocampus we have found no evidence that the A₁ receptor effects could be modified by inhibi-

tors of protein kinases (A or C). Previous investigators have also demonstrated a lack of relationship between cAMP levels and the electrophysiological effects of adenosine [14–17], except in forskolin-treated hippocampal slices [18].

Since there is evidence for an interaction between adenosine and nitric oxide [19–21], we have examined the effects of the competitive nitric oxide synthase inhibitor L-NAME and the brain-specific inhibitor 7-nitroindazole, but have found that neither showed any effects on the inhibition of potentials produced by CPA. These results indicate that nitric oxide does not play a significant role in the inhibition produced by A₁ adenosine receptors in the hippocampus.

However, it is well established that, postsynaptically, adenosine increases potassium conductance [22–24], and barium can block selectively the postsynaptic hyperpola-

Fig. 5. **A** Intracellularly elicited action potentials in the presence of CPA or CPA + CGS21680, superimposed to indicate that there was no difference in the spike shape induced by these compounds. **B** Records overlaid to coincide at the start of the depolarising pulse, revealing the increased spike threshold induced by CPA and the restoration to the control level (not shown separately) by CGS21680.

rising effects of adenosine [25–28]. In this study we have shown that the E-S dissociation caused by adenosine A_1 receptor activation studied extracellularly, and the directly measured effect on spike threshold recorded intracellularly, are also prevented by barium or glibenclamide. The possibility exists that a similar suppression of a potassium current may be the mechanism by which adenosine A_{2A} receptor activation causes inhibition of A_1 receptor-mediated changes of spike threshold.

Barium can block several potassium currents [29, 30], and it is not yet clear which of these could be involved in the A_1/A_{2A} receptor interaction. It is unlikely that the IA current is involved, however, as Pan et al. [31] have shown

that, whereas barium blocks the postsynaptic hyperpolarisation induced by adenosine, it does not prevent adenosine activation of the A-current. On the other hand, the additional finding that glibenclamide can prevent the postsynaptic but not presynaptic effects of CPA suggests that the ATP-sensitive channels may be involved, as proposed in earlier work [32].

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