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Influence of metabotropic glutamate receptor agonists on the inhibitory effects of adenosine A_1 receptor activation in the rat hippocampus

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1 Glutamate and other amino acids are the main excitatory neurotransmitters in many brain regions, including the hippocampus, by activating ion channel-coupled glutamate receptors, as well as metabotropic receptors linked to G proteins and second messenger systems. Several conditions which promote the release of glutamate, like frequency stimulation and hypoxia, also lead to an increase in the extracellular levels of the important neuromodulator, adenosine. We studied whether the activation of different subgroups of metabotropic glutamate receptors (mGluR) could modify the known inhibitory effects of a selective adenosine A_1 receptor agonist on synaptic transmission in the hippocampus. The experiments were performed on hippocampal slices taken from young (12–14 days old) rats. Stimulation was delivered to the Schaffer collateral/commissural fibres, and evoked field excitatory postsynaptic potentials (fe.p.s.p.) recorded extracellularly from the stratum radiatum in the CA1 area.

2 The concentration-response curve for the inhibitory effects of the selective adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA; 2–50 nM), on the fe.p.s.p. slope (EC_{50} = 12.5 (9.2–17.3; 95% confidence intervals)) was displaced to the right by the group I mGluR selective agonist, (**R,S**)-3,5-dihydroxyphenylglycine (DPHG; 10 μM) (EC_{50} = 27.2 (21.4–34.5) nM, n = 4). The attenuation of the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope by DHPG (10 μM) was blocked in the presence of the mGluR antagonist (which blocks group I and II mGluR), (**R,S**)-α-methyl-4-carboxyphenylglycine (MCPG; 500 μM). DHPG (10 μM) itself had an inhibitory effect of 20.1 ± 1.9% (n = 4) on the fe.p.s.p. slope.

3 The concentration-response curves for the inhibitory effects of CPA (2–20 nM) on the fe.p.s.p. slope were not modified either in the presence of the group II mGluR selective agonist, (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I; 1 μM), or in the presence of the non-selective mGluR agonist (which activates both group I and II mGluR), (1**S**,3**R**)-1-aminocyclopentyl-1,3-dicarboxylate (ACPD; 100 μM). L-CCG-I had no consistent effects and ACPD (100 μM) decreased by 19.4 ± 1.8% (n = 4) the fe.p.s.p. slope.

4 The concentration-response curve for the inhibitory effects of CPA (2–100 nM) on the fe.p.s.p. slope (EC_{50} = 8.2 (6.9–9.6) nM) was displaced to the right by the group III mGluR selective agonist, L-2-amino-4-phosphonobutyrate (L-AP4; 25 μM) (EC_{50} = 17.7 (13.1–21.9) nM, n = 4). The attenuation of the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope by L-AP4 (25 μM) was blocked in the presence of the mGluR antagonist (selective for the group III mGluR), (**R,S**)-α-methyl-4-phosphonophenylglycine (MPPG; 200 μM).

5 Both the direct effect of DHPG on synaptic transmission and the attenuation of the inhibitory effect of CPA (10 nM) were prevented in the presence of the protein kinase C selective inhibitors, staurosporine (1 μM) or chelerythrine (5 μM), and thus attributed to activation of protein kinase C.

6 The attenuation by L-AP4 (25 μM) of the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope was also prevented by the protein kinase C selective inhibitors, staurosporine (1 μM) or chelerythrine (5 μM), and thus attributed to activation of protein kinase C. But this effect seemed to be distinct from the direct effect of L-AP4 (25 μM) on synaptic transmission, which was not modified by the protein kinase C selective inhibitors.

7 We conclude that agonists of metabotropic glutamate receptors (Groups I and III) are able to attenuate the inhibitory effects of adenosine A_1 receptor activation in the hippocampus. This interaction may have pathophysiological relevance in hypoxia, in which there is marked release of both excitatory amino acids and the important endogenous neuroprotective substance, adenosine.

Keywords: Metabotropic glutamate receptor (mGluR); 3,5-dihydroxyphenylglycine (DHPG); (1**S**,3**R**)-1-aminocyclopentyl-1,3-dicarboxylate (ACPD); L-AP4; adenosine A_1 receptor; protein kinase C; hippocampus

Introduction

Glutamate and other amino acids are the main excitatory neurotransmitters in many brain regions, including the hippocampus, and activate ion channel-coupled glutamate receptors. These excitatory amino acids also activate other type of receptors, metabotropic glutamate receptors (mGluR), which are linked to G proteins and second messenger systems (for review see Knöpfel *et al*., 1995). At least 8 mGLuR subtypes have been cloned and they can be divided into 3 groups based on sequence homology, transducing mechanisms involved and pharmacological characteristics. Group I mGluR (mGluR1 and mGluR5) couple via phospholipase C to the inositol triphosphate/Ca^{2+} signalling pathway in expression systems and are selectively activated by (**R,S**)-3,5-dihydroxy-

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phenylglycine (DHPG). Group II mGluR (mGluR2 and mGluR3) couple negatively to adenylyl cyclase in expression systems and are selectively activated by (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I) and (2**S**,1′**R**,2′**R**,3′**R**)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV). Group III mGluR (mGluR4, mGluR6, mGluR7 and mGluR8) also inhibit adenylyl cyclase in expression systems and are selectively activated by L-2-amino-4-phosphonobutyrate (L-AP4).

It is interesting that several conditions which promote the release of glutamate also lead to an increase in the extracellular levels of the important neuromodulator, adenosine. This is the case with frequency stimulation (Mitchell *et al*., 1993) and hypoxia or ischaemia (Berne *et al*., 1974). Several aspects of the interactions between excitatory amino acid transmission mediated through ionotropic glutamate receptors and adenosine have previously been studied (see de Mendonça & Ribeiro, 1993), but the possibility of interactions between metabotropic glutamate receptors and adenosine has not been studied. We thus examined whether activation of different subtypes of mGluR could modify the known inhibitory effects of a selective adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA), on synaptic transmission in the hippocampus. We used the group I mGluR selective agonist, (**R,S**)-3,5-dihydroxyphenylglycine (DHPG; 10 μM) (Ito *et al*., 1992; Schoepp *et al*., 1994), the group II mGluR selective agonist, (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I; 1 μM) (Hayashi *et al*., 1992), the non-selective mGluR agonist (which activates both group I and II mGluR), (1**S**, 3**R**)-1-aminocyclopentyl-1,3-dicarboxylate (ACPD; 100 μM) (Watkins and Collingridge, 1994) and the group III mGluR selective agonist, L-2-amino-4-phosphonobutyrate (L-AP4; 25 μM) (Nakanishi, 1992; Thomsen *et al*., 1992), as well as the non-selective mGluR antagonist (which blocks mainly group I and II mGluR), (**R,S**)-α-methyl-4-carboxyphenylglycine (MCPG; 500 μM) (Thomsen *et al*., 1994; Hayashi *et al*., 1994; Cavanni *et al*., 1994) and the group III mGluR selective antagonist, (**R,S**)-α-methyl-4-phosphonophenylglycine (MPPG; 200 μM) (Jane *et al*., 1995).

Methods

The experiments were performed on hippocampal slices taken from young (12–14 days old) Wistar rats, since the effects of mGluR activation are usually more pronounced in young animals (Baskys & Malenka, 1991). The animals were anaesthetized with halothane, decapitated, and the right hippocampus dissected free in ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgSO_4$ 1, $CaCl_2$ 2, glucose 10, and gassed with a 95% O_2 + 5% CO_2 mixture. Slices (400 μm thick) were cut perpendicularly to the long axis of the hippocampus with a McIlwain tissue chopper, and kept in a resting chamber within the same gassed medium at room temperature (22–25°C). After about one hour, individual slices were eventually transferred to the submerged recording chamber (1 ml capacity), where they were continuously superfused at a rate of 3 ml min^{-1} with the same gassed solution at 30.5°C. Drugs were added to this superfusing solution. Stimulation was delivered to the Schaffer collateral/commissural fibres by bipolar concentric wire electrodes placed in stratum radiatum under transillumination of the slice. Rectangular pulses of 0.1 ms duration were applied once every 15 s. The initial intensity of the stimulus was that eliciting an evoked field excitatory postsynaptic potential (fe.p.s.p.) of about 1 mV amplitude, without appreciable contamination by the population spike. The fe.p.s.ps were recorded extracellularly from CA1 stratum radiatum by use of micropipettes filled with 4 M NaCl and of 2–4 MΩ resistance, and displayed on a Tektronix digitizing oscilloscope. The averages of 8 consecutive responses were obtained, graphically plotted and recorded for further analysis with a personal computer by use of locally developed software. The fe.p.s.ps were quantified both as the peak amplitude and the slope of the initial phase of the potential. Since the effects were found to be similar with both methods, only the fe.p.s.p. slope data are shown.

The drugs used were: (**R,S**)-3,5-dihydorxyphenylglycine (DHPG), (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I), (1**S**,3**R**)-1-amino-cyclopentyl-1,3-dicarboxylate (ACPD), L-2-amino-4-phosphonobutyrate (L-AP4), (**R,S**)-α-methyl-4-phosphonophenylglycine (MPPG), (**R,S**)-α-methyl-4-carboxyphenylglycine (MCPG) (all from Tocris Cookson); staurosporine (Sigma); Rp-adenosine-3′,5′-cyclic monophosphorothioate (Rp-cyclic AMPS), chelerythrine (both from Research Biochemicals International). N^6-cyclopentyladenosine (CPA) was made up as a 10 mM stock solution in dimethylsulphoxide (DMSO) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) made up as a 5 mM stock solution in DMSO 99% v/v containing 0.01 M NaOH (both from Research Biochemicals International).

Values are usually given as the means ± s.e.mean. When two successive cumulative concentration-response curves were obtained for the inhibitory effects of CPA, care was taken that the initial magnitudes of the fe.p.s.ps were similar, by adjusting stimulus intensity. This was the case when using mGluR agonists which had by themselves inhibitory effects on the slope of the fe.p.s.p. Furthermore, these mGluR receptor agonists were applied at concentrations that had inhibitory effects not exceeding 20% of fe.p.s.p. decrease. The curve fitting and the EC_{50} (with 95% confidence intervals) were obtained by use of the Prism software (Graphpad Software, Inc.). The EC_{50} values were considered significantly different when the 95% confidence intervals were exclusive. The significance of the differences between two means was calculated by use of Student's *t* test, paired or non-paired, as appropriate. The significance of the differences between more than two means obtained in the same slices was calculated with repeated-measures ANOVA followed by the Newman-Keuls test, by use of the Prism software. *P* values less than 0.05 were considered statistically significant. In the figures these statistical differences are represented as asterisks, and the s.e.mean as vertical lines.

Results

Effects of the group I mGluR selective agonist, DHPG, on the inhibitory effect of CPA

We first determined that under control conditions two successive concentration-response curves for the inhibitory effects of the adenosine A_1-selective agonist, N^6-cyclopentyladenosine (CPA; 2–20 nM), on the fe.p.s.p. slope were superimposable (Figure 1a), an EC_{50} of 11.9 (10.7–13.3) nM being obtained for the first curve and an EC_{50} of 12.6 (11.0–14.4) nM for the second curve ($n=3$, a non-significant difference). We then found that the concentration-response curve for the inhibitory effects of CPA (2–50 nM) on the fe.p.s.p. slope was displaced to the right by the group I mGluR selective agonist, (**R,S**)-3,5-dihydroxyphenylglycine (DHPG; 10 μM) (Figure 1b). An EC_{50} of 12.5 (9.2–17.3) nM was found for the inhibitory effect of CPA on the fe.p.s.p. slope in the control solution which was significantly different from the EC_{50} of 27.2 (21.4–34.5) nM determined in the same experiments ($n=4$) for the inhibitory effect of CPA on the fe.p.s.p. slope in the presence of DHPG (10 μM). It should be noted that DHPG (10 μM) itself had an inhibitory effect of 20.1 ± 1.9% ($n=4$) on the fe.p.s.p. slope.

The DHPG-induced attenuation of the inhibitory effect of CPA is mediated by metabotropic glutamate receptors

The attenuation of the inhibitory effect of CPA (10 nM; a single concentration close to the EC_{50} was used) on the fe.p.s.p. slope by DHPG (10 μM) (Figure 1c) was blocked by the mGluR antagonist (which blocks mainly group I and II mGluR), (**R,S**)-α-methyl-4-carboxyphenylglycine (MCPG; 500 μM) (Figure 1d). The inhibitory effect of CPA (10 nM) in

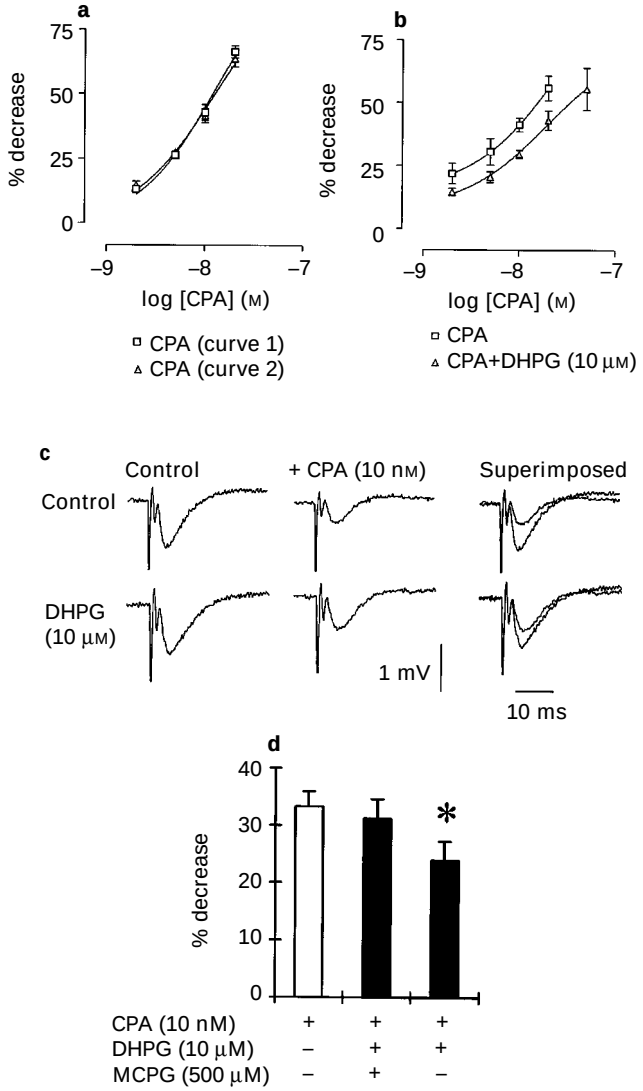

Figure 1 The group I mGluR selective agonist, (**R,S**)-3,5-dihydroxyphenylglycine (DHPG; 10 μM), reduced the inhibitory effect of the adenosine A_1 receptor selective agonist, N^6-cyclopentyladenosine (CPA), on synaptic transmission. (a) Under control conditions two successive concentration-response curves for the inhibitory effects of CPA (2–20 nM) on the fe.p.s.p. slope were superimposable ($n=3$). (b) In the presence of DHPG (10 μM) the concentration-response curve for the inhibitory effects of CPA (2–50 nM) on the fe.p.s.p. slope was displaced to the right ($n=4$). (c) Representative fe.p.s.p. (each an average of 8 consecutive responses) from a single experiment illustrating the inhibitory effect of CPA (10 nM) under control conditions (upper traces) and the attenuation of this effect in the presence of DHPG (10 μM) (lower traces). (d) In the presence of the mGluR antagonist (which blocks mainly group I and II mGluR), (**R,S**)-α-methyl-4-carboxyphenylglycine (MCPG; 500 μM), DHPG (10 μM) could no longer attenuate the inhibitory effects of CPA (10 nM), but this ability was restored after washing out the mGluR antagonist ($n=3$). *$P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test, the inhibitory effect of CPA (10 nM) in the presence of DHPG (10 μM) was significantly different from the inhibitory effect of CPA (10 nM) alone, and from the inhibitory effect of CPA (10 nM) in the presence of both DHPG (10 μM) and MCPG (500 μM).

the presence of both DHPG (10 μM) and MCPG (500 μM), 31.2±3.4%, was not different from the inhibitory effect of CPA (10 nM) alone, 33.3±2.7%, and both were significantly different from the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope in the presence of DHPG (10 μM), 23.9±3.3%, all these effects being studied in the same slices ($n=3$; $P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test) (Figure 1d). In the same experiments, the direct inhibitory effect of DHPG (10 μM) on synaptic transmission was also blocked by MCPG (500 μM), a slight inhibitory effect of 0.6±1.9% ($n=3$) being observed in the presence of MCPG (500 μM), as compared to the inhibitory effect of 20.1±1.9% ($n=4$) in the control solution (see above) ($P<0.05$, non-paired Student's t test).

The effects of DHPG are not mediated by inhibitory adenosine receptors

In order to exclude the possibility that the group I mGluR selective agonist, DHPG (10 μM), modified the inhibitory effects of CPA on the fe.p.s.p. slope by activating adenosine receptors, we tested whether the inhibitory effects of DHPG (10 μM) on the fe.p.s.p. slope could be prevented by the selective adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), in a concentration (10 nM) that is about 20 times the K_i value, and about 4 times the concentration that antagonizes completely the maximal effect of an adenosine A_1 receptor agonist, determined in electrophysiological studies in the hippocampus (Sebastião *et al*., 1990). In the same slices, DHPG (10 μM) decreased the fe.p.s.p. slope by 14.3±2.0% under control conditions (because successive applications of DHPG (10 μM) caused smaller inhibitory effects on the fe.p.s.p. slope we took the average of the first and third applications of DHPG (10 μM) in the control solution), and by 14.1±2.2% ($n=3$; a non-significant difference) in the presence of DPCPX (10 nM; determined during the second application of DHPG (10 μM); DPCPX was subsequently washed out for more than 90 min).

Effects of the group II mGluR selective agonist, L-CCG-I, on the inhibitory effect of CPA

The concentration-response curve for the inhibitory effects of CPA (2–20 nM) on the fe.p.s.p. slope was not modified in the presence of the group II mGluR selective agonist, (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I; 1 μM) (Figure 2a). An EC_{50} of 13.1 (10.0–16.7) nM was found for the inhibitory effect of CPA on the fe.p.s.p. slope which was not significantly different from the EC_{50} of 15.1 (12.7–18.1) nM determined in the same experiments ($n=4$) for the inhibitory effect of CPA on the fe.p.s.p. slope in the presence of L-CCG-I (1 μM). At this concentration (1 μM), L-CCG-I had no consistent effects on the fe.p.s.p. slope. It should be noted that higher concentrations of L-CCG-I were not used, since activation of metabotropic glutamate receptors other than group II might occur (Hayashi *et al*., 1992).

Effects of the mGluR non-selective agonist, ACPD, on the inhibitory effect of CPA

The concentration-response curve for the inhibitory effects of CPA (2–20 nM) on the fe.p.s.p. slope was not modified in the presence of the non-selective mGluR agonist (which activates both group I and II mGluR), (1**S**,3**R**)-1-aminocyclopentyl-1,3-dicarboxylate (ACPD; 100 μM) (Figure 2b). An EC_{50} of 8.3 (7.2–9.6) nM was found for the inhibitory effect of CPA on the fe.p.s.p. slope which was not significantly different from the EC_{50} of 8.4 (6.9–10.3) nM determined in the same experiments ($n=4$) for the inhibitory effect of CPA on the fe.p.s.p. slope in the presence of ACPD (100 μM). ACPD (100 μM) itself had an inhibitory effect of 19.4±1.8% ($n=4$) on the fe.p.s.p. slope.

Effects of the group III mGluR selective agonist, L-AP4, on the inhibitory effect of CPA

The concentration-response curve for the inhibitory effects of CPA (2–100 nM) on the fe.p.s.p. slope was displaced to the right by the group III mGluR selective agonist, L-2-amino-

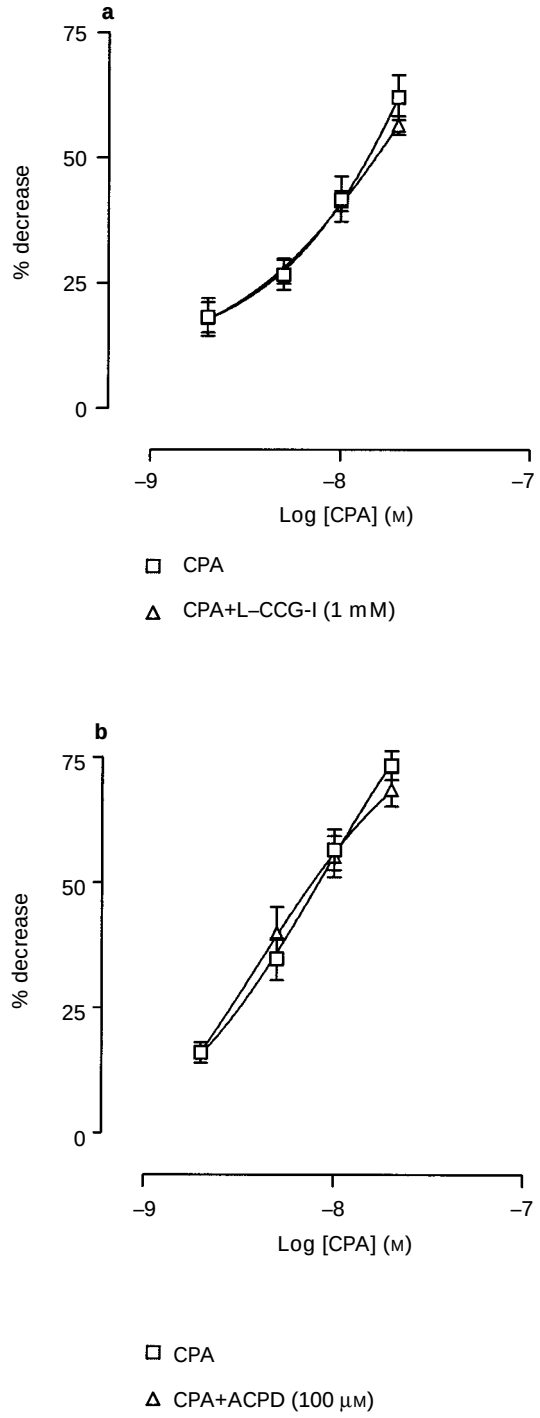

Figure 2 Both the group II mGluR selective agonist, (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I; 1 μM; $n=4$) (a), and the non-selective mGluR agonist (1**S**,3**R**)-1-aminocyclopentyl-1,3-dicarboxylate (ACPD; 100 μM; $n=4$) (b), did not appreciably modify the concentration-response curve for the inhibitory effects of the adenosine A_1 receptor selective agonist, N^6-cyclopentyladenosine (CPA; 2–20 nM) on the fe.p.s.p. slope.

4-phosphonobutyrate (L-AP4; 25 μM) (Figure 3a). An EC_{50} of 8.2 (6.9–9.6) nM was found for the inhibitory effect of CPA on the fe.p.s.p. slope in the control solution which was significantly different from the EC_{50} of 17.7 (13.1–21.9) nM determined in the same experiments ($n=4$) for the inhibitory effect of CPA on the fe.p.s.p. slope in the presence of L-AP4 (25 μM). It should be noted that L-AP4 (25 μM) itself had an inhibitory effect of $25.2\pm6.2\%$ ($n=4$) on the fe.p.s.p. slope.

The L-AP4-induced attenuation of the inhibitory effect of CPA is mediated by specific metabotropic glutamate receptors

The attenuation of the inhibitory effect of CPA (10 nM; a single concentration close to the EC_{50} was used) on the fe.p.s.p. slope by L-AP4 (25 μM) (Figure 3b) was blocked in the presence of the mGluR antagonist (selective for the group III mGluR), (**R,S**)-α-methyl-4-phosphonophenylglycine (MPPG; 200 μM) (Figure 3c). The inhibitory effect of CPA (10 nM) in the presence of both L-AP4 (25 μM) and MPPG (200 μM), $34.5\pm1.5\%$, was not different from the inhibitory effect of CPA (10 nM) alone, $36.1\pm3.6\%$, and both were significantly different from the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope in the presence of L-AP4 (25 μM), $26.0\pm1.7\%$; all these effects were studied in the same slices ($n=4$; $P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test) (Figure 3c). In the same experiments, the direct inhibitory effect of L-AP4 (25 μM) on synaptic transmission was if anything only partially blocked by MPPG (200 μM), an inhibitory effect of $15.1\pm2.9\%$ ($n=4$) being observed in the presence of MPPG (200 μM), as compared to the inhibitory effect of $25.2\pm6.2\%$ ($n=4$) in the control solution (see above) (a non-significant difference).

The effects of L-AP4 are not mediated by inhibitory adenosine receptors

In order to exclude the possibility that the group III mGluR selective agonist, L-2-amino-4-phosphonobutyrate (L-AP4; 25 μM) modifies the inhibitory effects of CPA on the fe.p.s.p. slope by activating adenosine receptors, we tested whether the inhibitory effects of L-AP4 (25 μM) on the fe.p.s.p. slope could be prevented by an adenosine A_1 receptor antagonist. This was not the case since, in the same slices, L-AP4 (25 μM) decreased the fe.p.s.p slope by $19.1\pm2.6\%$ under control conditions and by $19.8\pm2.1\%$ ($n=3$; a non-significant difference) in the presence of the selective adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 10 nM). The mGluR antagonist, MPPG, has been shown previously not to interfere with adenosine receptors (Bushell *et al.*, 1996).

The DHPG-induced attenuation of the inhibitory effect of CPA involves a protein kinase C-dependent mechanism

The attenuation of the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope by DHPG (10 μM) was prevented by the protein kinase C relatively-selective inhibitor, staurosporine (1 μM) (Figure 4a). The inhibitory effect of CPA (10 nM) in the presence of both DHPG (10 μM) and staurosporine (1 μM), $42.5\pm1.2\%$, was not significantly different from the inhibitory effect of CPA (10 nM) alone, $43.2\pm1.6\%$, or the inhibitory effect of CPA (10 nM) in the presence of staurosporine (1 μM), $41.1\pm1.4\%$, but these effects were significantly different from the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope in the presence of DHPG (10 μM), $36.8\pm0.7\%$ (all effects were determined in the same slices, $n=3$, $P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test) (Figure 4a). The inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope was thus not modified by staurosporine (1 μM), but the attenuation of this inhibitory effect of CPA (10 nM) by DHPG (10 μM) was prevented by staurosporine (1 μM) and thus dependent on protein kinase C transducing mechanisms. Similar mechanisms appear to be involved in the inhibitory effect of DHPG (10 μM) on the fe.p.s.p. slope since, as mentioned above, DHPG (10 μM) inhibited by $20.1\pm1.9\%$ ($n=4$) the fe.p.s.p. slope, whereas in the presence of staurosporine (1 μM) an inhibition of only $8.7\pm1.4\%$ ($n=3$; $P<0.05$, non-paired Student's t test) was observed.

In order to confirm these results, in further experiments we used the more selective protein kinase C inhibitor, chelerythrine. Again we found that the attenuation of the inhibitory effect

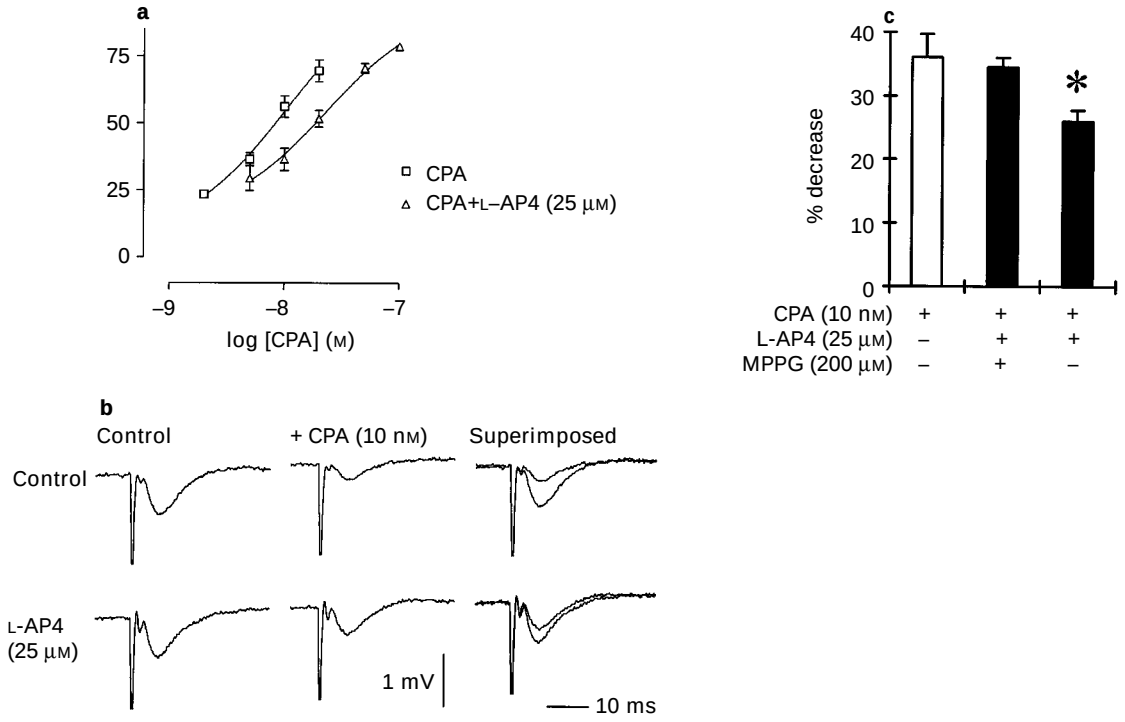

Figure 3 The group III mGluR selective agonist, L-2-amino-4-phosphonobutyrate (L-AP4; 25 μM), reduced the inhibitory effect of the adenosine A_1 receptor selective agonist, N^6-cyclopentyladenosine (CPA), on synaptic transmission. (a) The concentration-response curve for the inhibitory effects of CPA (2–100 nM) on the fe.p.s.p. slope was displaced to the right in the presence of L-AP4 (25 μM; $n=4$). (b) Representative fe.p.s.p. (each an average of 8 consecutive responses) from a single experiment illustrating the inhibitory effect of CPA (10 nM) under control conditions (upper traces) and the attenuation of this effect in the presence of L-AP4 (25 μM) (lower traces). (c) In the presence of the mGluR antagonist (selective for the group III mGluR), (**R,S**)-α-methyl-4-phosphonophenylglycine (MPPG; 200 μM), L-AP4 (25 μM) could no longer attenuate the inhibitory effects of CPA (10 nM), but this ability was restored after washing out the mGluR selective antagonist ($n=4$). *$P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test, the inhibitory effect of CPA (10 nM) in the presence of L-AP4 (25 μM) was significantly different from the inhibitory effect of CPA (10 nM) alone, and from the inhibitory effect of CPA (10 nM) in the presence of both L-AP4 (25 μM) and MPPG (200 μM).

of CPA (10 nM) on the fe.p.s.p. slope by DHPG (10 μM) was prevented in the presence of this protein kinase C-selective inhibitor (Figure 4b). The inhibitory effect of CPA (10 nM) in the presence of both DHPG (10 μM) and chelerythrine (5 μM), $51.1\pm6.9\%$, was not significantly different from the inhibitory effect of CPA (10 nM) alone, $51.1\pm9.5\%$, or the inhibitory effect of CPA (10 nM) in the presence of chelerythrine (5 μM), $51.9\pm8.9\%$, but these effects were significantly different from the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope in the presence of DHPG (10 μM), $39.0\pm5.4\%$ (all effects were determined in the same slices, $n=3$, $P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test) (Figure 4b).

The L-AP4-induced attenuation of the inhibitory effect of CPA also involves a protein kinase C-dependent mechanism

The attenuation of the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope by L-AP4 (25 μM) was prevented by the protein kinase C relatively selective inhibitor, staurosporine (1 μM) (Figure 4c). The inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope in the presence of both L-AP4 (25 μM) and staurosporine (1 μM), $46.5\pm6.0\%$, was not significantly different from the inhibitory effect of CPA (10 nM) alone, $47.7\pm7.3\%$, or the inhibitory effect of CPA (10 nM) in the presence of staurosporine (1 μM), $44.2\pm6.4\%$, but these were significantly different from the inhibitory effect of CPA (10 nM) in the presence of L-AP4 (25 μM), $33.3\pm3.5\%$ (all effects being determined in the same slices, $n=3$, $P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test) (Figure 4c). As previously determined, the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope was not modified by staurosporine (1 μM) (see Figure 4a), but the attenuation of the inhibitory effect of CPA (10 nM) by L-AP4 (25 μM) was prevented by staurosporine (1 μM), and thus dependent on protein kinase C transducing mechanisms. However, in this case a different mechanism appears to be involved in the direct inhibitory effect of L-AP4 (25 μM) on the fe.p.s.p. slope, since L-AP4 (25 μM) inhibited by $25.2\pm6.2\%$ ($n=4$) the fe.p.s.p. slope under control conditions, as shown above, and by $24.6\pm0.7\%$ ($n=3$) in the presence of staurosporine (1 μM) (a non-significant difference). By including data from all experiments performed with staurosporine (1 μM), this compound elicited only a slight change in the fe.p.s.p. slope of $2.9\pm3.3\%$ ($n=6$).

Using chelerythrine, we confirmed that the attenuation of the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope by L-AP4 (25 μM) was dependent upon protein kinase C (Figure 4d). The inhibitory effect of CPA (10 nM) in the presence of both L-AP4 (25 μM) and chelerythrine (5 μM), $56.9\pm1.9\%$, was not significantly different from the inhibitory effect of CPA (10 nM) alone, $55.7\pm2.0\%$, or the inhibitory effect of CPA (10 nM) in the presence of chelerythrine (5 μM), $59.7\pm2.7\%$, but these effects were significantly different from the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope in the presence of L-AP4 (25 μM), $47.0\pm1.3\%$ (all effects were determined in the same slices, $n=3$, $P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test) (Figure 4d). By taking all experiments performed with chelerythrine (5 μM), this compound elicited only a slight change in the fe.p.s.p. slope of $-2.1\pm3.3\%$ ($n=6$).

In some further experiments we tested another protein kinase inhibitor, Rp-adenosine-3′,5′-cyclic monophosphorothioate (Rp-cyclic AMPS), which is selective for protein kinase A. In contrast to the inhibition of protein kinase C, which did not

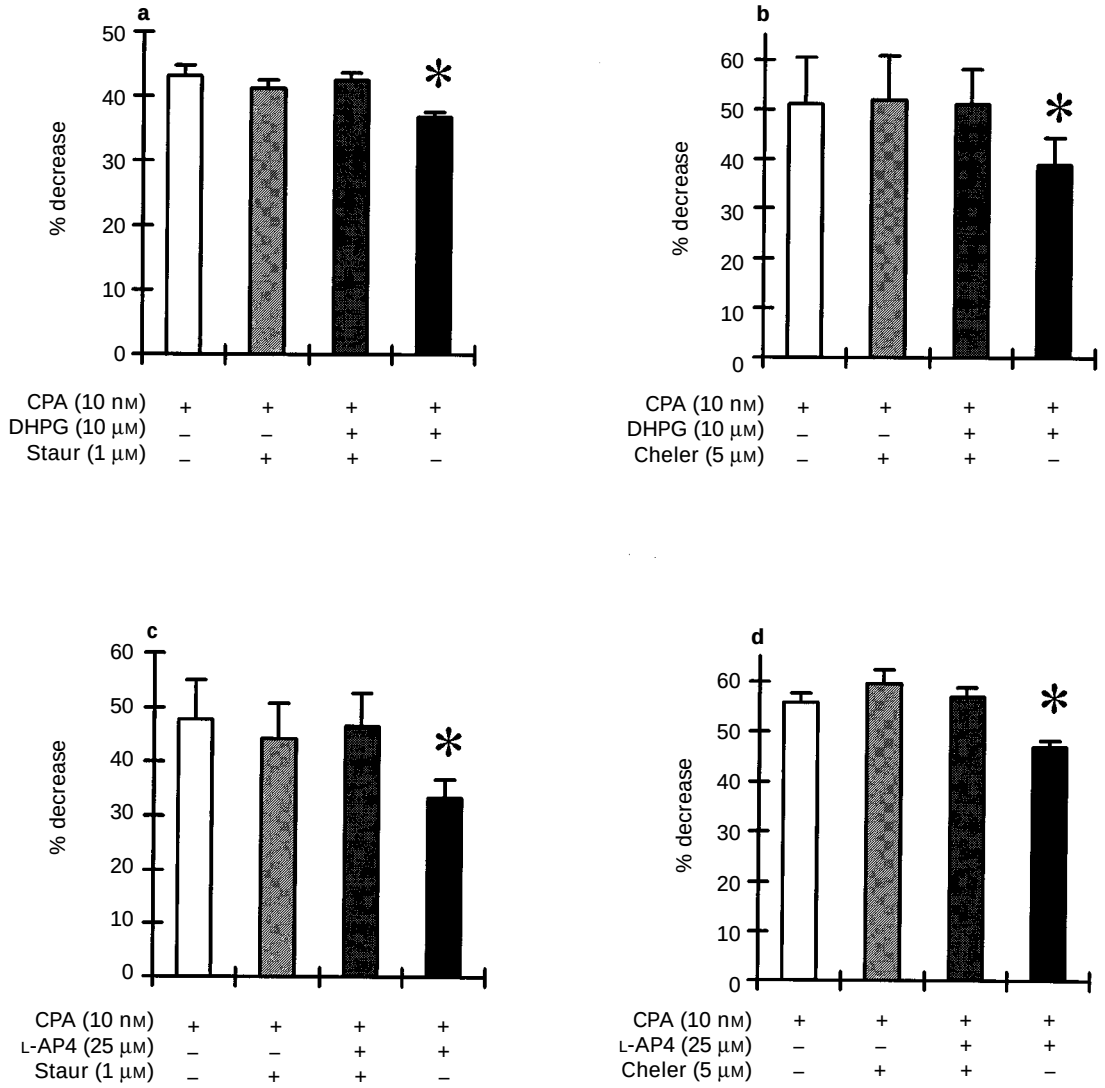

Figure 4 Both the group I mGluR selective agonist, (**R,S**)-3,5-dihydroxyphenylglycine (DHPG) and the group III mGluR selective agonist, L-2-amino-4-phosphonobutyrate (L-AP4; 25 μM), attenuated the inhibitory effect of the adenosine A_1 receptor selective agonist, N^6-cyclopentyladenosine (CPA), on synaptic transmission by protein kinase C-dependent mechanisms. DHPG (10 μM) could not attenuate the inhibitory effects of CPA (10 nM) on the fe.p.s.p. slope in the presence of the protein kinase C inhibitors, staurosporine (1 μM; $n=3$) (a) or chelerythrine (5 μM; $n=3$) (b), but this effect of DHPG (10 μM) was restored after washing out the protein kinase C inhibitor (as seen in the last column). In a similar way, L-AP4 (25 μM) could not attenuate the inhibitory effects of CPA (10 nM) on the fe.p.s.p.slope in the presence of the protein kinase C inhibitors, staurosporine (1 μM; $n=3$) (c) or chelerythrine (5 μM; $n=3$) (d), but this effect of L-AP4 (25 μM) was restored after washing out the protein kinase C inhibitor (as seen in the last column). $*P<0.05$, repeated measures ANOVA followed by the Newman-Keuls test, the inhibitory effect of CPA (10 nM) in the presence of the mGluR agonist (DHPG (10 μM) or L-AP4 (25 μM)) was significantly different from the inhibitory effect of CPA (10 nM) alone, from the inhibitory effect of CPA (10 nM) in the presence of the protein kinase C inhibitor (staurosporine (1 μM) or chelerythrine (5 μM)), and from the inhibitory effect of CPA (10 nM) in the presence of both the mGluR agonist and the protein kinase C inhibitor.

modify the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope, Rp-cyclic AMPS itself could attenuate the inhibitory effect of CPA (10 nM) on the fe.p.s.p. slope. The inhibitory effect of CPA (10 nM) in the control solution was $44.3\pm4.8\%$, whereas in the presence of Rp-cyclic AMPS (50 μM) a value of $33.7\pm2.2\%$ was obtained ($n=3$, $P<0.05$, paired Student's t test).

Discussion

The group I mGluR selective agonist, DHPG, inhibited synaptic transmission at the Schaffer fibre-CA1 synapse, as shown previously (Gereau & Conn, 1995; Manzoni & Bockaert, 1995). We showed that DHPG could attenuate the inhibitory effects of the selective adenosine A_1 receptor agonist, CPA, on synaptic transmission. Both effects of the group I mGluR selective agonist seemed to involve activation of protein kinase C, since they were prevented in the presence of the protein kinase C relatively-selective inhibitor, staurosporine, or the more selective inhibitor, chelerythrine (Herbert *et al.*, 1990). However, due to the incomplete degree of selectivity of these inhibitors, the relatively high concentrations used, and the complexity of protein kinases in the brain, the involvement of other protein kinases cannot be excluded. It is known that the group I mGluR couple positively to phospholipase C, promoting phosphoinositide hydrolysis and thus generating inositol 1,4,5-triphosphate as well as diacylglycerol, which activates protein kinase C (Schoepp *et al.*, 1994). Furthermore, activation of protein kinase C is able to attenuate the presynaptic inhibitory effects of adenosine in the hippocampus (Thompson *et al.*, 1992), as well as at the neuromuscular junction (Sebastião & Ribeiro, 1990), and suppresses the inhibitory action of a selective adenosine A_1 receptor agonist on

glutamate release in cerebrocortical synaptosomes (Budd & Nicholls, 1995). We may thus advance the hypothesis that the group I mGluR selective agonist, DHPG, attenuated the inhibitory effects of CPA on synaptic transmission by a protein kinase C-dependent mechanism. It should be noted that, in contrast to what happens when protein kinase C is activated exogenously, the inhibitory effect of CPA on synaptic transmission was not appreciably modulated by endogenous protein kinase C, the inhibitory effect of CPA being similar in either the presence or absence of staurosporine, or chelerythrine. In contrast, the inhibitory effect of CPA on synaptic transmission appeared to be regulated by endogenous protein kinase A activation, since it was attenuated in the presence of the protein kinase A selective inhibitor, Rp-cyclic AMPS (van Haastert *et al*., 1984). However, we found it difficult to ascertain whether Rp-cyclic AMPS actually contributed to the attenuation of the CPA inhibitory effect by L-AP4, since it caused a modification in the effect of CPA itself.

The group II mGluR selective agonist, L-CCG-I, did not appreciably modify synaptic transmission at the Schaffer fibre-CA1 synapse, in accordance with previous data obtained with this (Manzoni & Bockaert, 1995) and another group II mGluR selective agonist, (2**S**,1′**R**,2′**R**,3′**R**)-2-(2,3-dicarboxycyclopropyl)glycine (DGC-IV) (Gereau & Conn, 1995) in the same hippocampal area. This contrasts with the known inhibitory effects of group II mGluR selective agonists at the mossy fibre-CA3 (Manzoni *et al*., 1995) and both medial and lateral perforant path-dentate gyrus synapses (Ugolini & Bordi, 1995; Bushell *et al*., 1996). We observed that the group II mGluR selective agonist, L-CCG-I, did not modify the inhibitory effects of the selective adenosine A_1 receptor agonist, CPA, on synaptic transmission. Similarly, the non-selective mGluR agonist, ACPD, in spite of being found previously to inhibit synaptic transmission in the CA1 area (Baskys & Malenka, 1991; Manzoni & Bockaert, 1995; Vignes *et al*., 1995), did not appreciably modify the inhibitory effects of CPA. A possible interpretation is that, since ACPD is non-selective, it activates not only group I mGluR but also other mGluR, namely from group II (Watkins & Collingridge, 1994).

The group III mGluR selective agonist, L-AP4, also inhibited synaptic transmission at the Schaffer fibre-CA1 synapse, as shown previously (Baskys & Malenka, 1991; Manahan-Vaughan & Reymann, 1995; Manzoni & Bockaert 1995; Gereau & Conn, 1995). This effect is known to be presynaptic, since L-AP4 increases paired-pulse facilitation, does not modify the responses elicited by direct application of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and reduces the frequency but not the amplitude of spontaneous miniature excitatory postsynaptic currents (me.p.s.cs) (Baskys & Malenka, 1991; Gereau & Conn, 1995). Accordingly, L-AP4 inhibits the Ca^{2+}-dependent release of glutamate in cerebrocortical synaptosomes from young animals (Vázquez *et al*., 1995). Furthermore, L-AP4 does not exert noticeable postsynaptic effects on CA1 pyramidal cells (Davies *et al*., 1995). In the present study, we observed that the group III mGluR selective agonist, L-AP4, attenuated the inhibitory effects of the selective adenosine A_1 receptor agonist, CPA, on synaptic transmission. This modulation of the effects of CPA seemed to be distinct from the direct inhibitory action of L-AP4 on the fe.p.s.p. slope. In fact, the direct inhibitory effect of L-AP4 on the fe.p.s.p. slope did not involve activation of protein kinase C, since it was not modified in the presence of the protein kinase C selective inhibitors, staurosporine or chelerythrine. Also note that the inhibiton of glutamate release by L-AP4 in cerebrocortical synaptosomes is insensitive to staurosporine (Herrero *et al*., 1996; see review by Sánchez-Prieto *et al*., 1996). In contrast, the attenuation of the inhibitory effects of CPA by L-AP4 was prevented by either staurosporine or chelerythrine, and thus found to be dependent upon protein kinase C activation. Besides, the attenuation of the inhibitory effects of CPA by L-AP4 was completely prevented by the metabotropic glutamate receptor selective antagonist, MPPG (200 μM), but the direct inhibitory effect of L-AP4 on synaptic transmission was if anything only partially blocked by MPPG at this concentration, which may suggest that these effects are mediated through different metabotropic glutamate receptors.

We propose that the attenuation of the inhibitory effects of adenosine caused by activation of metabotropic glutamate receptors might have pathophysiological relevance in situations, such as hypoxia and ischaemia, in which marked release of both excitatory amino acids (Benveniste *et al*., 1984) and adenosine (Berne *et al*., 1974) occurs. It is possible that the excessive release of glutamate would limit the known neuroprotective effects of adenosine (Rudolphi *et al*., 1992). This possibility should be taken in account when designing therapeutic strategies that use metabotropic glutamate receptors as targets for the treatment of neurological disorders (Knöpfel *et al*., 1996).

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