ELSEVIER

European Journal of Pharmacology 340 (1997) 35–44

ejp

Group II and III metabotropic glutamate receptors modulate paired pulse depression in the rat dentate gyrus in vitro

Deirdre M. O'Leary, Eugene M. Cassidy [1], John J. O'Connor *

Department of Human Anatomy and Physiology, University College, Earlsfort Terrace, Dublin 2, Ireland

Received 13 March 1997; revised 6 October 1997; accepted 7 October 1997

Abstract

We have investigated the effect of a number of group I, II and III metabotropic glutamate (mGlu) receptor agonists and antagonists on paired pulse depression in the medial perforant path of the rat dentate gyrus in vitro. A triphasic pattern of a large depression at short intervals (10–50 ms), a reduction of this depression at intermediate intervals (50–200 ms) and again a large depression at late intervals (> 200 ms) was observed. The group I mGlu receptor agonist, (*S*)-3,5-dihydroxy phenylglycine ((*S*)-DHPG; 20 μM) had no significant effect on paired pulse depression at any interstimulus intervals. The mGlu receptor group II and III agonists, L-CCG-1 ((2*S*,3*S*,4*S*)-α-(carboxy–cyclopropyl)-glycine), DCG-IV ((2*S*,1′*R*,2′*R*,3′*R*)-2-2′,3′-dicarboxy cyclopropylglycine), 1*S*,3*R*-ACPD (1*S*,3*R*-1-aminocyclopentate-1,3-dicarboxylic acid) and L-AP4 (L-2-amino-4-phosphono butyric acid) reduced paired pulse depression at interstimulus intervals of 200 ms or less. Application of the non specific mGlu receptor antagonist, MCPG (α-methyl carboxy–phenylglycine; 200 μM) completely inhibited the 1S,3R ACPD-induced reduction in paired pulse depression but was without effect on the L-AP4 response. The relatively specific group II antagonist MCCG ((2*S*,3*S*,4*S*)-2-methyl-2-carboxy cycloproprylglycine) at 200 μM and 500 μM, attenuated but did not completely inhibit the DCG-IV induced reduction of paired pulse depression. The putative group III pre-synaptic mGlu receptor antagonist α-methyl-L-AP4 and MSOP ((*RS*)-α-methylserine-*O*-phosphate) both at 200 μM inhibited the L-AP4-induced reduction in paired pulse depression at intermediate phase interstimulus intervals but not at early interstimulus intervals. These results specifically demonstrate the involvement of group II and III mGlu receptor ligands in the modulation of paired pulse depression in the medial perforant pathway. © 1997 Elsevier Science B.V.

Keywords: Paired pulse depression; mGlu receptor; 1*S*,3*R*-ACPD (1*S*,3*R*-1-aminocyclopentate-1,3-dicarboxylic acid); DCG-IV ((2*S*,1′*R*,2′*R*,3′*R*)-2-2′,3′-dicarboxy cyclopropylglycine); MCPG (α-methyl carboxy–phenylglycine); L-AP4 (L-2-amino-4-phosphonobutyric acid); α-Methyl-L-AP4 (α-methyl-L-amino-4-phosphonobutyric acid); Hippocampal slice

1. Introduction

The perforant path input to the hippocampus can be divided anatomically and physiologically into the lateral and medial components (McNaughton, 1980). These distinct pathways can be differentiated according to their response to paired pulse stimulation and to stimulation of pre-synaptic metabotropic glutamate (mGlu) receptors (Kahle and Cotman, 1993a). Several forms of synaptic plasticity have been described in the perforant path of the hippocampus including long term potentiation (Bliss and Lomo, 1973), paired pulse facilitation (Harris and Cotman, 1985; Kahle and Cotman, 1993a) and paired pulse depression (McNaughton, 1980).

Stimulation of the medial perforant path leads to a depression of the second synaptic response to paired stimuli at short- (10–20 ms) and long-latency (200–2000 ms) interstimulus intervals separated by a period of reduced depression at intermediate interstimulus intervals (50–200 ms; DiScenna and Teyler, 1994), whereas paired pulse stimulation in the lateral perforant path (LPP) has produced variable results (McNaughton, 1980). This triphasic pattern in the medial perforant path is evident in animals from an early age onwards (DiScenna and Teyler, 1994) unlike long-term potentiation which has been shown to be reduced during ageing. Such short term synaptic plasticity

* Corresponding author. Tel.: +353-1-7067263; fax: +353-1-7067417; e-mail: john.oconnor@ucd.ie.

[1] Present address: Department of Psychiatry, Cluain Mhuire Family Centre, Hospitaller Order of St. John of God, Newtown park Avenue, Blackrock, Co Dublin, Ireland.

may also be part of the mechanism of hippocampal processing of sensory inputs that enter via the perforant path.

Paired pulse depression of the second synaptic response to paired stimuli in the medial perforant pathway of the dentate gyrus has been well characterised across a broad range of interstimulus intervals both in vivo and in vitro (Harris and Cotman, 1985; Kahle and Cotman, 1993a,b). There is now evidence that mGlu receptors modulate perforant path responses, via both pre- and post-synaptic mechanisms in the hippocampus (Kahle and Cotman, 1993b; Brown and Reymann, 1995). These receptors are coupled to a variety of second-messenger systems via GTP-binding proteins (Schoepp et al., 1990; Conn et al., 1994).

To date, eight different mGlu receptor clones have been isolated from rat cDNA libraries ($mGlu_{1-8}$ receptors) and have been classified into three groups based upon similarities in their effector coupling, sequence homology and agonist selectivity (Schoepp and Conn, 1993; Pin and Duvoisin, 1995). There is also evidence for a mGlu receptor subtype distinct from any known to date, which is linked to phospholipase D and facilitates transmitter release (Boss and Conn, 1992; Pellegrini-Giampietro et al., 1996).

The first group (group I) comprising $mGlu_1$ receptor (with its four splice variants) and $mGlu_5$ receptor (with its two splice variants) are coupled to phosphoinositide hydrolysis. In addition the $mGlu_1$ receptor has been shown to stimulate cAMP and arachidonic acid (Aramori and Nakanishi, 1992). Group II, comprising the $mGlu_2$ receptor and $mGlu_3$ receptor, and group III, comprising the $mGlu_{4,6-8}$ receptor, all inhibit the accumulation of cAMP (for example see Okamoto et al., 1994).

Group II mGlu receptors can be differentiated from group III receptors by their sensitivity to (L)-2-amino-4-phosphonobutanoic acid (L-AP4), which acts as an agonist at group III receptors (Watkins and Collingridge, 1994). Glutamate transmission has been shown to be inhibited pre-synaptically by L-AP4 in many brain regions including the hippocampus (Trombley and Westbrook, 1992). The actions of L-AP4 have been shown to be inhibited by (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid (α-methyl-L-AP4), a novel putative selective antagonist at pre-synaptic L-AP4 receptors (Bushell et al., 1995), although α-methyl-L-AP4 has recently been reported to act with low affinity at group II mGlu receptors (Gomeza et al., 1996). The different pharmacological profiles for the various subtypes of mGlu receptors suggest that they are functionally distinct within the central nervous system. While 1*S*,3*R*-ACPD, DCG-IV and L-AP4 have been demonstrated to be selective agonists for the mGlu receptor family, they are not subtype specific. Also, no mGlu receptor antagonist to date has been reported to be specific and potent at any single receptor subtype.

The role which mGlu receptors play in short and long-term plasticity remains controversial. In the present study we have therefore investigated the effect of a number of non-specific and specific mGlu receptor agonists and antagonists on paired pulse depression in the medial perforant pathway of the rat dentate gyrus in vitro. We have also investigated any differential effects of these compounds on early, intermediate and late phase paired pulse depression. Some of these results have previously appeared in abstract form (Cassidy and O'Connor, 1995; O'Leary and O'Connor, 1997).

2. Materials and methods

2.1. Animals and solution

All experiments were carried out on transverse slices of the hippocampus of the rat (weight 50–100 g) by standard methods (O'Connor et al., 1994). Briefly, brains were rapidly removed after decapitation and placed in cold oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF; concentrations in mM; NaCl, 120; KCl 2.5, NaH_2P0_4, 1.25; $NaHC0_3$ 26; $MgS0_4$, 2.0; $CaCl_2$, 1.5; D-glucose 10). Slices were cut at a thickness of 350 μm using a Camden vibroslice, and transferred to a storage container containing oxygenated medium at room temperature. The slices were then transferred as required to a recording chamber for submerged slices, and continuously perfused at a rate of 5–7 ml/min with oxygenated medium at 30–32°C.

2.2. Stimulation and recording

A glass stimulating electrode was placed in the outer two-thirds of the molecular layer of the dentate gyrus to stimulate the pre-synaptic afferent fibres entering the hippocampal formation in the medial perforant pathway. Stimulation was carried out with a Grass S48 stimulator every 20 s (intensity, 5–15 V; duration, 0.1 ms) to evoke field excitatory postsynaptic potentials (EPSPs) in the dentate granule cell dendrites of 30–50% maximal amplitude EPSP. Paired pulses were applied at interstimulus intervals of 10, 50, 100, 200, 500 and 800 ms. The recording electrode was placed in the middle third of the dentate molecular layer to record paired population granule cell dendritic postsynaptic responses to medial perforant pathway stimulation. Evoked responses were amplified using a Grass P16 microelectrode amplifier and displayed on standard storage oscilloscope (Tektronix Instruments). Recordings were analysed off-line using the Strathclyde electrophysiology software (Dr. J. Dempster).

At a given interstimulus interval, paired pulse depression was calculated as $(S1 - S2)/S1$ percent where $S1$ and $S2$ represent the amplitudes of the first and second responses to paired stimuli respectively. Paired pulse depression in the presence of drugs was expressed as a percentage of control paired pulse depression values. In

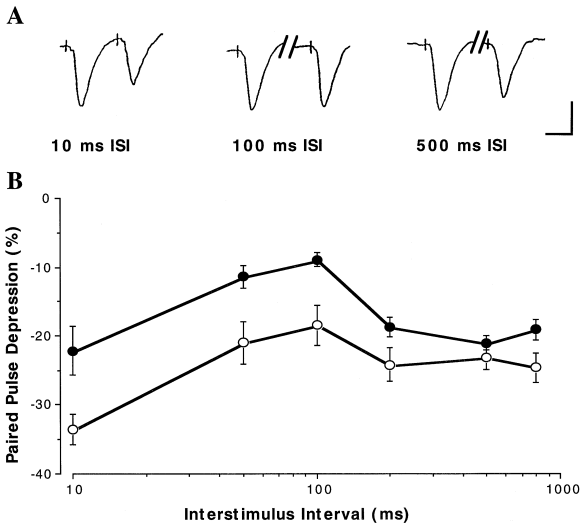

Fig. 1. Characterisation of paired pulse depression in the medial perforant path of the dentate gyrus. Three separate phases can be seen. (A) Typical traces show representative paired pulse depression at interstimulus intervals of 10, 100 and 500 ms in normal ACSF (1.5 mM Ca^{2+}). In this slice a large depression of *S*2 (2nd EPSP) amplitude is visible at early (10 ms) and late (500 ms) interstimulus intervals, while a reduced depression is seen at intermediate intervals (100 ms). (B) Averaged data showing the paired pulse depression observed at the different interstimulus intervals in normal (1.5 mM; ●) and elevated external Ca^{2+} (3 mM; ○). Three phases can be seen at both concentrations (10–40 ms; 50–200 ms and $>$ 200 ms). There was a significant increase in paired pulse depression in elevated Ca^{2+} at all interstimulus intervals (Student's *t*-test, unpaired; $P < 0.05$ for all interstimulus intervals). Each point in control ACSF is the average of 28 observations while in elevated external Ca^{2+} the average of 8 observations is shown for each point.

experiments where paired pulse facilitation was observed, values are also expressed as a percentage of control paired pulse depression values. For the sake of clarity paired pulse depression has been expressed as a negative value while paired pulse facilitation has been expressed as a positive value. During paired stimuli EPSP slope measurements were also carried out since a recent report by Schulz et al. (1995) has indicated some discrepancies in the measurement of paired pulse facilitation depending on whether amplitude or slope was used. We found no significant differences in our results (data not shown). In experiments where mGlu receptor ligands decreased baseline EPSP slope, the stimulation voltage was raised occasionally to increase the EPSP slope to pre-drug control levels and paired pulse depression was measured. Each condition was tested in at least 4 (specific *n* noted) slices taken from different animals and recorded on different days. Summarised results are expressed as mean ± S.E.M. Data were analysed statistically with the paired and unpaired (Fig. 1B) two-tailed Student's *t*-test.

2.3. Drugs

Drugs used were L-2-amino-4-phosphonobutyric acid (L-AP4), α-methyl-L-AP4, 1*S*,3*R*-1-aminocyclopentate-1,3-dicarboxylic acid (1*S*,3*R*-ACPD), α-methyl-4-carboxy–phenyl glycine (MCPG), (2*S*,1′*R*,2′*R*,3′*R*)-2-2′,3′-dicarboxycyclopropyl glycine (DCG-IV), (2*S*,3*S*,4*S*)-2-methyl-2-carboxycyclopropylglycine (MCCG), (*RS*)-α-methylserine-*O*-phosphate (MSOP), (*S*)-3,5-dihydroxy phenylglycine ((*S*)-DHPG), (*S*)-4-carboxyphenylglycine (4-CPG), (2*S*,3*S*,4*S*)-α-(carboxy–cyclopropyl)–glycine (L-CCG-1), (*RS*)-α-methylserine-*O*-phosphate monophenyl ester (MSOPPE) all obtained from Tocris Cookson. All other chemicals were obtained from Sigma Chemical. All drugs were bath applied to the slices as part of the perfusate during recording for at least 30 min except 1*S*,3*R*-ACPD where in 4 extra experiments it was applied for 10 min. No significant differences were observed and

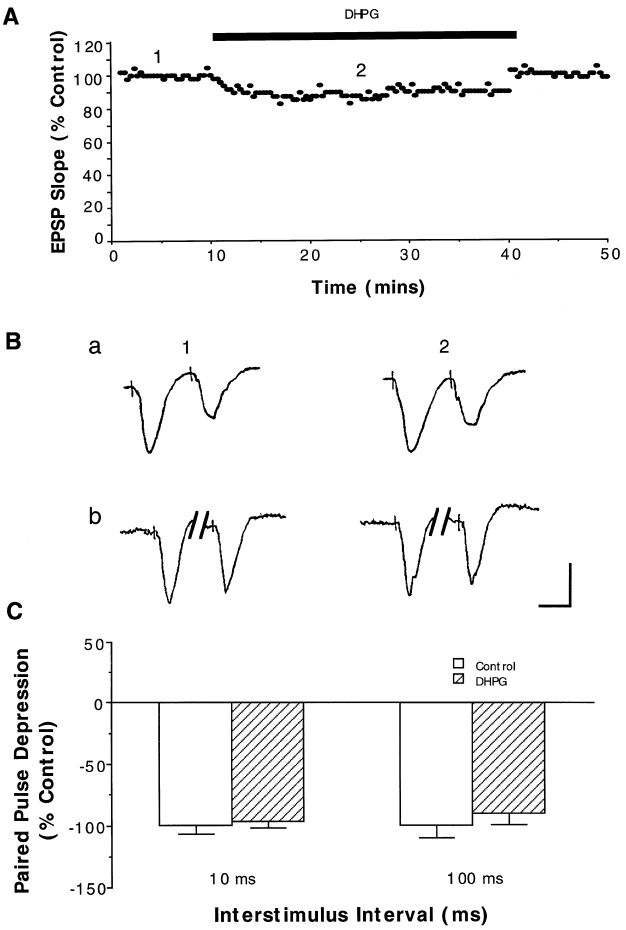

Fig. 2. Effect of (*S*)-DHPG on baseline EPSP slope and paired pulse depression in the medial perforant path of the dentate gyrus. (A) Typical experiment showing the time course of the effects of (*S*)-DHPG on EPSP slope. (*S*)-DHPG (20 μM) was applied for 30 min causing a reversible reduction in EPSP slope. (B) Typical examples of traces showing paired pulses applied at intervals of 10 ms (a) and 100 ms (b) at the times indicated in (A). 1, control; 2, in the presence of (*S*)-DHPG. Horizontal bar, 5 ms; vertical bar, 0.5 mV. (C) Averaged data for the effect of (*S*)-DHPG on paired pulse depression at interstimulus intervals of 10 and 100 ms. (*S*)-DHPG had no significant effect on paired pulse depression at all interstimulus intervals (diagonally striped bars). Each bar is the mean ± S.E.M. ($n = 8$).

the data were pooled. Data were recorded before drug application, during drug application and in some cases after washout with ACSF. No mGlu receptor agonist had a significant effect on paired pulse depression at interstimulus intervals greater than 200 ms in our experiments and for clarity this data is not included.

3. Results

3.1. Control paired pulse depression

Fig. 1A shows typical control responses from the medial perforant path when pairs of stimuli were delivered at 10, 50, 100, 200, 500 and 800 ms interstimulus intervals. The second synaptic response was consistently smaller than the first at early ($\leq$ 50 ms; e.g. $-22.1 \pm 3.5\%$ at 10 ms inter stimulus interval) and late phase paired pulse depression ($\geq$ 200 ms; e.g. $-21.1 \pm 1.0\%$ at 500 ms inter stimulus interval; $n = 28$). A small period of reduced depression is also evident in control slices at interstimulus intervals between 50 and 200 ms (e.g. $-9.0 \pm 1.0\%$ at 100 ms interstimulus interval; Fig. 1A and B). Increasing the extracellular calcium concentration to 3 mM increased paired pulse depression at all interstimulus intervals tested (e.g. $-33.6 \pm 2.2\%$ at 10 ms interstimulus interval; $P < 0.05$; $n = 8$; Fig. 1B).

3.2. Effect of group I mGlu receptor ligands on EPSP slope and paired pulse depression

The mGlu receptor group I agonist, (*S*)-DHPG (20 μM), significantly reduced the baseline EPSP slope (92.0 $\pm$ 1.0% of control; $n = 5$; Fig. 2A; $P < 0.05$) but had no significant effect on paired pulse depression at all interstimulus intervals tested (Fig. 2B and C). However, the group I antagonist, 4-CPG (200 μM) when applied on its own reduced baseline EPSP slope ($80.0 \pm 1.0\%$ of control; $n = 5$; $P < 0.05$) and attenuated paired pulse depression at both early and intermediate intervals ($n = 5$; $P < 0.05$; see Table 1). Combined application of 4-CPG and (*S*)-DHPG had no further effect on paired pulse depression at all intervals.

3.3. Effect of group II mGlu receptor ligands on EPSP slope and paired pulse depression

Application of the non selective mGlu receptor group II agonist, L-CCG-1 (20 μM) for 30 min had no effect on the baseline EPSP slope ($98.1 \pm 0.7\%$ of control; $n = 6$; Fig. 3A). However L-CCG-1 at 50 μM, significantly reduced baseline EPSP slope ($76.5 \pm 13.5\%$ of control; $P < 0.05$; $n = 3$; Fig. 3A). L-CCG-1 (20 μM) significantly attenuated paired pulse depression at early interstimulus intervals ($-27.5 \pm 16.4\%$ of control paired pulse depression; $n = 6$; $P < 0.05$) while at intermediate interstimulus intervals a reduction to $+2.9 \pm 31.4\%$ of control paired pulse depression was observed. ($n = 6$; $P < 0.05$; compared to control paired pulse depression; Fig. 3B and C). The group II antagonist (*RS*)-α-methylserine-*O*-phosphate monophenyl ester (MSOPPE) (200 μM) applied on its own significantly reduced the baseline EPSP slope to $83.7 \pm 1.3\%$ of control ($n = 4$; $P < 0.05$; data not shown) but had no significant effect on paired pulse depression at all interstimulus intervals tested ($n = 4$). MSOPPE did not reverse the effects of L-CCG-1 on paired pulse depression at any interval ($n = 5$; data not shown).

The group II agonist DCG-IV (1 μM) significantly reduced baseline EPSP slope ($33.8 \pm 4.4\%$ of control; $n = 4$; Fig. 4A; $P < 0.05$) when applied for 30 min. DCG-

Table 1
Effects of metabotropic glutamate receptor agonists and antagonists on paired pulse depression in the medial perforant path of the rat dentate gyrus in vitro

	Ligand	PPD (10 ms ISI)	PPD (100 ms ISI)
Group I	DHPG (20 μM)	$-97.1 \pm 4.8\%$ NS ($n = 8$)	$-89.7 \pm 9.3\%$ NS ($n = 8$)
	4-CPG (200 μM)	$-28.1 \pm 14.4\%$[a] ($n = 5$)	$-50.3 \pm 9.3\%$[a] ($n = 5$)
	4-CPG + DHPG (200 μM)	$-8.8 \pm 12.6\%$[a] ($n = 5$)	$-26.8 \pm 23.4\%$[a] ($n = 5$)
Group II	L-CCG-1 (20 μM)	$-27.5 \pm 16.4\%$[a] ($n = 5$)	$-2.9 \pm 31.4\%$[a] ($n = 5$)
	DCG-IV (1 μM)	$-1.3 \pm 12.1\%$[a] ($n = 4$)	$+91.6 \pm 28.2\%$[a] ($n = 4$)
	MCCG-1 + DCG-IV (200 μM)	$-55.0 \pm 2.9\%$[b] ($n = 4$)	$-20.9 \pm 17.1\%$[b] ($n = 4$)
	ACPD* (10 μM)	$-55.5 \pm 7.6\%$[a] ($n = 6$)	$-40.2 \pm 12.5\%$[a] ($n = 6$)
	MCPG + ACPD (200 μM)	$-106.6 \pm 8.0\%$ NS ($n = 6$)	$-106.3 \pm 21.6\%$ NS ($n = 6$)
	MAP-4 + ACPD (200 μM)	$-58.4 \pm 7.2\%$[a] ($n = 5$)	$-51.1 \pm 7.5\%$[a] ($n = 5$)
Group III	L-AP4 (20 μM)	$-61.3 \pm 9.5\%$[a] ($n = 9$)	$-47.2 \pm 6.0\%$[a] ($n = 9$)
	MAP-4 + L-AP4 (200 μM)	$-62.4 \pm 9.8\%$[a] ($n = 5$)	$-109.8 \pm 20.1\%$ NS ($n = 5$)
	MSOP + L-AP4 (200 μM)	$-72.4 \pm 15.2\%$[a] ($n = 4$)	$-112.2 \pm 4.8\%$ NS ($n = 4$)
	MCPG + L-AP4 (200 μM)	$-69.8 \pm 10.0\%$[a] ($n = 5$)	$-13.4 \pm 24.2\%$[a] ($n = 5$)

Values are expressed as a mean $\pm$ S.E.M. percentage of control paired pulse depression values (-100%). Negative values indicate paired pulse depression and positive values indicate paired pulse facilitation. Total n values and ligand concentrations are indicated in each case.
[a]Significantly different from control paired pulse depression values ($P < 0.05$; Student's paired *t*-test).
[b]Significantly different from agonist alone values ($P < 0.05$). NS; not significantly different from control paired pulse depression values.
*Also a group I agonist.

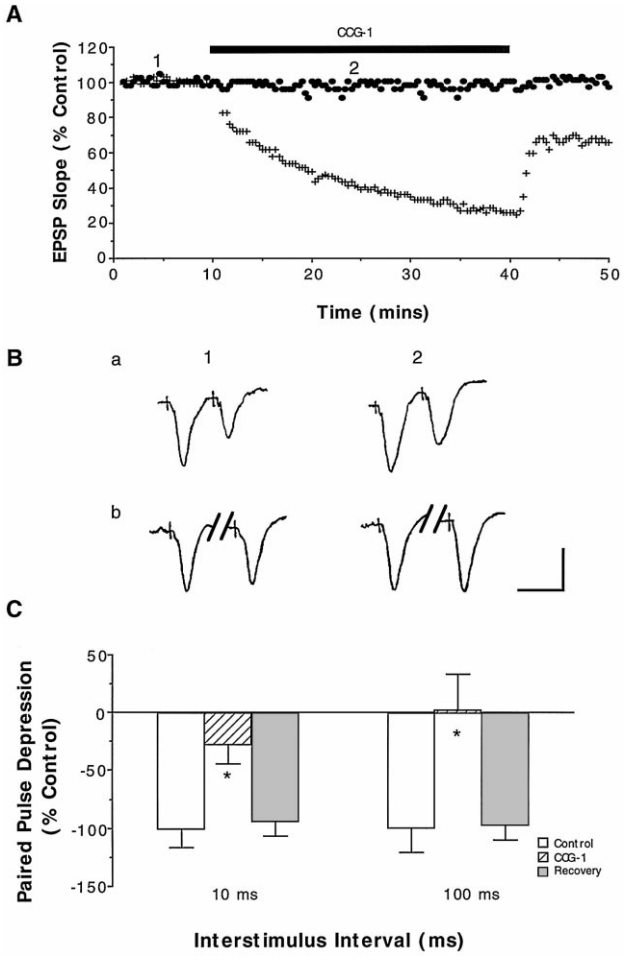

Fig. 3. Effect of L-CCG-1 on baseline EPSP slope and paired pulse depression in the medial perforant path of the dentate gyrus. (A) Typical experiment showing the time course of the effects of L-CCG-1 on baseline EPSP slope. Application of L-CCG-1 (20 μM; ●) for 30 min caused no significant reduction in EPSP slope. 50 μM L-CCG-1 (+) caused a significant reduction in EPSP slope (25% of control). (B) Typical examples of traces showing paired pulses applied at intervals of 10 ms (a) and 100 ms (b) at the times indicated in A. 1, control; 2 in the presence of 20 μM L-CCG-1. Horizontal bar, 5 ms; vertical bar, 0.5 mV. (C) Averaged data for the effect of L-CCG-1 on paired pulse depression at interstimulus intervals of 10 and 100 ms. L-CCG-1 caused a significant attenuation of paired pulse depression at both interstimulus intervals (diagonally striped bars; $^{*}P < 0.05$ for both interstimulus intervals; mean $\pm$ S.E.M.; $n = 6$; Student's t-test). Following washout of L-CCG-1 (grey bars), paired pulse depression returned to control values. No effect was seen at interstimulus intervals of 200 ms or more (results not shown).

IV significantly reduced paired pulse depression at early interstimulus interval (-1.3 ± 12.1% of control paired pulse depression; $n = 4$; $P < 0.05$; Fig. 4B and C) and produced a paired pulse facilitation ($+91.6 \pm 28.2$% of control paired pulse depression) at intermediate interstimulus interval. The effect of DCG-IV on paired pulse depression was independent of the size of $S1$ amplitude since the responses were compared at similar $S1$ amplitudes before and after drug intervention and no significant differences were observed ($n = 4$). Following washout of DCG-IV, paired pulse depression returned to control levels ($n = 4$). Application of the group II antagonist MCCG-1 (200 μM and 500 μM) alone did not alter baseline EPSP slope or paired pulse depression (Fig. 4A and C, 200 μM only). MCCG-1(200 μM and 500 μM) significantly attenuated the inhibitory effect of DCG-IV (1 μM) on paired pulse

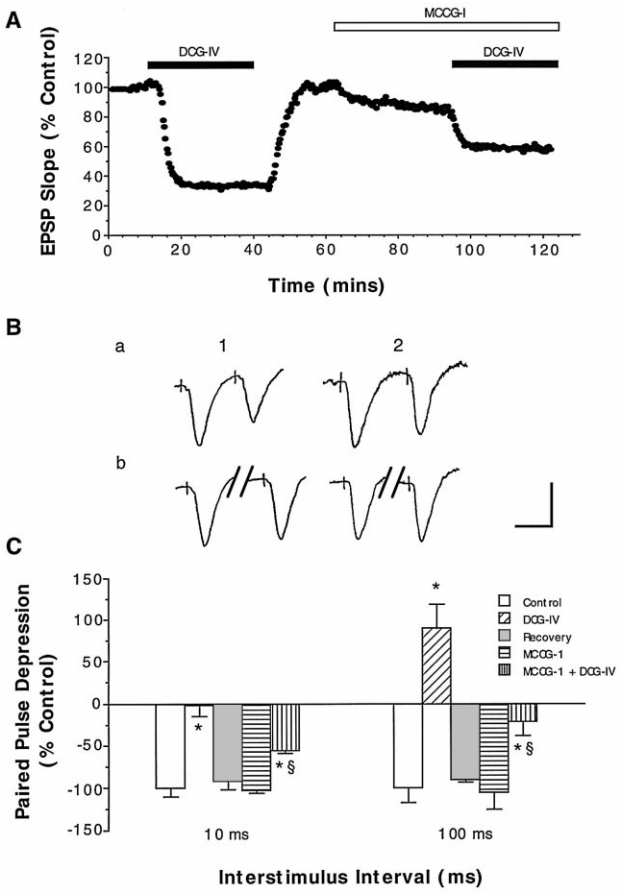

Fig. 4. Effect of DCG-IV and MCCG-1 on baseline EPSP slope and paired pulse depression in the medial perforant path of the dentate gyrus. (A) Typical experiment showing the time course of the effects of DCG-IV and MCCG-1 on baseline EPSP slope. Application of DCG-IV (1 μM) for 30 min reduced EPSP slope to 34% of control values which was reversible on washout. MCCG-1 (200 μM) had no significant effect on EPSP slope on its own and partially antagonised the effect of DCG-IV on the EPSP slope. (B) Typical examples of traces showing paired pulses applied at intervals of 10 ms (a) and 100 ms (b) at the times indicated in (A). 1, control; 2 in the presence of 1 μM DCG-IV. Horizontal bar, 5 ms; vertical bar, 0.5 mV. (C) Averaged data for the effect of DCG-IV and MCCG-1 on paired pulse depression at interstimulus intervals of 10 and 100 ms. DCG-IV significantly reduced paired pulse depression at early interstimulus intervals and unmasked a paired pulse facilitation at intermediate interstimulus intervals (diagonally striped bars; $^{*}P < 0.05$ for both compared to controls; mean $\pm$ S.E.M.; $n = 4$). Following washout of DCG-IV, paired pulse depression returned to control values ($n = 4$; grey bars). MCCG-1 (200 μM) had no significant effect on paired pulse depression at all interstimulus intervals when applied on its own (horizontally striped bars). However MCCG-1 significantly attenuated the inhibitory effect of DCG-IV on paired pulse depression at both 10 ms and 100 ms inter stimulus interval (vertically striped bars; $^{*}P < 0.05$ compared to controls; $^{\S}P < 0.05$ compared to DCG-IV alone; $n = 4$; Student's t-test). No effect was seen at interstimulus intervals of 200 ms or more (results not shown).

depression at early and intermediate interstimulus intervals (Fig. 4C; 200 μM only; $P < 0.05$ compared to DCG-IV alone). However, this was not a complete inhibition as paired pulse depression was still significantly different from control values (Fig. 4C; $n = 4$; $P < 0.05$).

3.4. Effect of group III mGlu receptor ligands on EPSP slope and paired pulse depression

Bath application of L-AP4 (2 and 20 μM) for 30 min in the medial perforant path was found to decrease *S*1 amplitude ($99.0 \pm 1.3\%$ and $78.1 \pm 1.7\%$ of control respectively; $n = 9$; $P < 0.05$ for 20 μM L-AP4 only; Fig. 5A). However, in the lateral perforant path, L-AP4 (20 μM) had a greater inhibitory effect on EPSP slope ($18.5 \pm 5.6\%$ of control; $n = 4$, data not shown). In the medial perforant path, L-AP4 at both concentrations reduced paired pulse depression at interstimulus intervals less than 500 ms (e.g. at 20 μM, $-61.3 \pm 9.5\%$ and $-47.2 \pm 6.0\%$ of control paired pulse depression at 10 and 100 ms interstimulus interval respectively; $n = 9$; $P < 0.05$; Fig. 5B and C). The effect of L-AP4 on paired pulse depression was independent of *S*1 amplitude as paired responses were also compared before and after drug intervention at similar *S*1 amplitudes with no differences observed ($n = 4$). Following washout of L-AP4, paired pulse depression returned to control values ($n = 4$).

The putative pre-synaptic group III mGlu receptor antagonist α-methyl-L-AP4 (200 μM and 500 μM) had no significant effect on baseline EPSP slope or paired pulse depression on its own ($n = 6$). α-Methyl-L-AP4 at 200 μM did not block the L-AP4 induced reduction in baseline EPSP slope ($n = 3$; Fig. 4A). α-Methyl-L-AP4 (500 μM) significantly reversed the inhibitory effect of L-AP4 on baseline EPSP slope (L-AP4, $75.1 \pm 2.6\%$ of control versus α-methyl-L-AP4 + L-AP4, $94.5 \pm 3.2\%$ of control; $n = 4$; $P < 0.05$). α-Methyl-L-AP4 at 200 μM and 500 μM reversed the L-AP4 effects on paired pulse depression at intermediate interstimulus intervals only (Fig. 4B; 200 μM only). It did not reverse the L-AP4 effect at 10 ms interstimulus interval ($P < 0.05$; $n = 5$; Fig. 5B).

Application of the putative group III antagonist MSOP (200 μM) alone had no effect on baseline EPSP slope (results not shown) or paired pulse depression. It did however inhibit the effects of L-AP4 at intermediate phase interstimulus intervals (Fig. 5C; $n = 4$; $P < 0.05$ for L-AP4 versus MSOP + L-AP4). Similar to α-methyl-L-AP4, MSOP did not reverse the effect of L-AP4 on paired pulse depression at 10 ms interstimulus interval ($P < 0.05$ for controls versus MSOP + L-AP4; $n = 4$; Fig. 5C).

3.5. Effect of 1*S*,3*R*-ACPD on the EPSP amplitude and paired pulse depression

Bath application of 1*S*,3*R*-ACPD (10 μM) for either 10 or 30 min was found to reduce *S*1 amplitude to $62.0 \pm 2.4\%$ of control ($n = 10$ pooled results; $P < 0.05$; Fig. 6A).

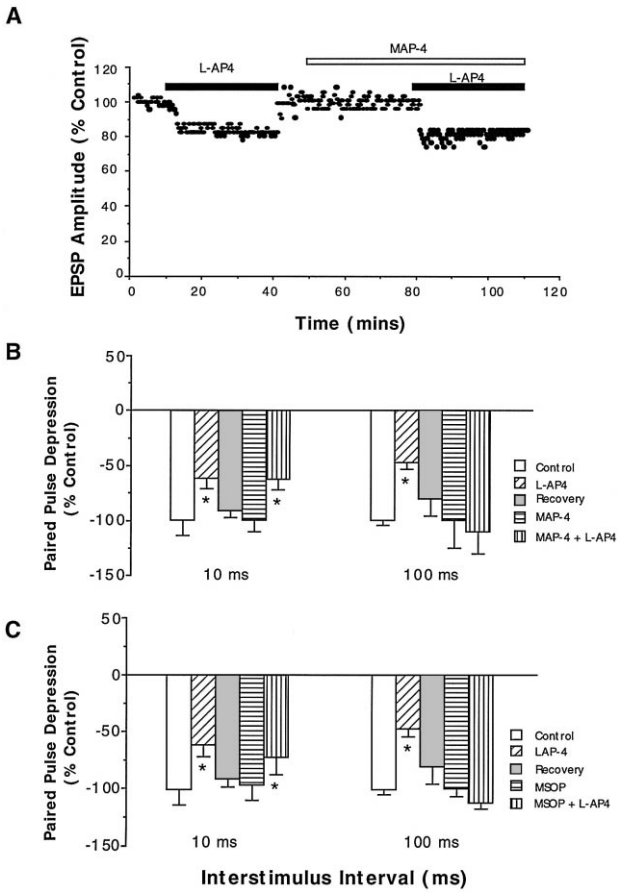

Fig. 5. Effect of group III mGlu receptors ligands on baseline EPSP slope and paired pulse depression in the medial perforant path of the dentate gyrus. (A) Typical experiment showing the time course of the effects of L-AP4 and α-methyl-L-AP4 (MAP-4) on EPSP slope. Application of L-AP4 (20 μM) for 30 min reduced EPSP slope (78% of control). Application of α-methyl-L-AP4 (200 μM) on its own had no effect on EPSP slope. (B) Averaged data for the effect of L-AP4 and α-methyl-L-AP4 on paired pulse depression at interstimulus intervals of 10 and 100 ms. L-AP4 (20 μM) reduced paired pulse depression at interstimulus intervals of 10 and 100 ms (diagonally striped bars) but not 500 ms (not shown). Following washout of L-AP4, paired pulse depression returned to control values ($n = 5$; grey bars). α-Methyl-L-AP4 (200 μM) had no significant effect on paired pulse depression on its own at all interstimulus intervals tested (horizontally striped bars), nor did it affect the L-AP4 actions at 10 ms inter stimulus interval (vertically striped bars). It did significantly inhibit the effect of L-AP4 at 100 ms ($^{*}P < 0.05$; compared to controls; $n = 5$; Student's *t*-test). (C) Averaged data for the effect of MSOP on the response to L-AP4 at 10 and 100 ms inter stimulus interval is shown. A new set of control (white bars), L-AP4 (diagonally striped bars) and recovery experiments (grey bars) were carried out ($n = 4$). MSOP had no effect on its own (horizontally striped bars) nor did it inhibit the effect of L-AP4 on early paired pulse depression (vertically striped bars). However, it did significantly inhibit the effect of L-AP4 at 100 ms ($^{*}P < 0.05$; $n = 4$; Student's *t*-test). Each bar is the mean ± S.E.M.

1*S*,3*R*, ACPD also reduced paired pulse depression at all interstimulus intervals less than 500 ms ($-55.5 \pm 7.6\%$ and $-40.2 \pm 12.5\%$ of control paired pulse depression at 10 and 100 ms interstimulus interval respectively; $n = 6$; $P < 0.05$; Fig. 6B). The effect of 1*S*,3*R*-ACPD on paired pulse depression was independent of *S*1 amplitude as

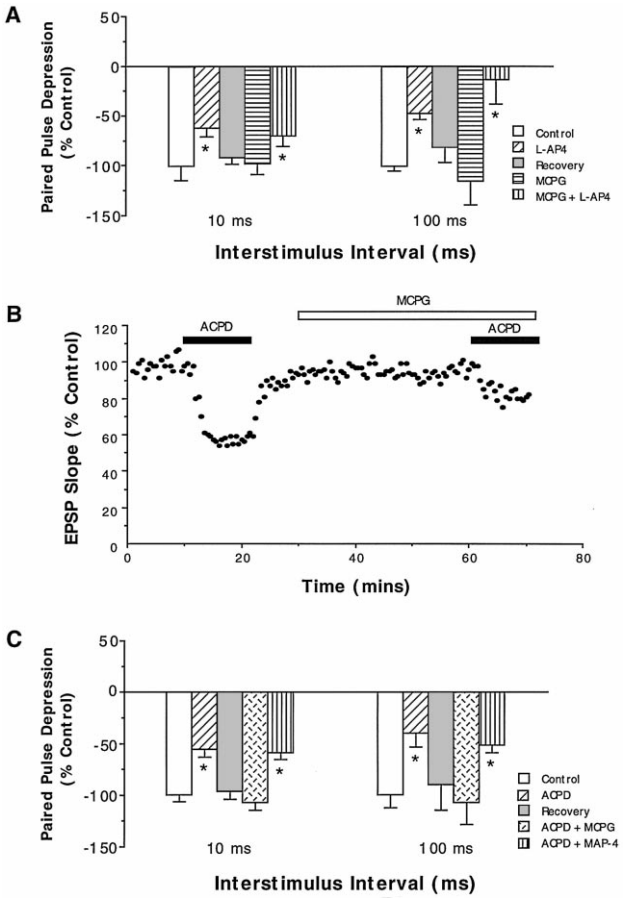

Fig. 6. Effect of 1*S*,3*R*-ACPD on baseline EPSP slope and paired pulse depression in the medial perforant path of the dentate gyrus. (A) Averaged data for the effect of MCPG on the response to L-AP4 at 10 and 100 ms inter stimulus interval is shown. A new set of control (white bars), L-AP4 (diagonally striped bars) and recovery experiments (grey bars) were carried out ($n = 4$). MCPG had no effect on its own (horizontally striped bars) nor did it inhibit the effect of L-AP4 on early or intermediate paired pulse depression (vertically striped bars; $^{*}P < 0.05$; $n = 5$). Late phase paired pulse depression was unaffected. (B) Typical experiment showing the time course of the effects of 1*S*,3*R*-ACPD and MCPG on EPSP slope. Application of 1*S*,3*R*-ACPD (20 μM) for 10 min reduced EPSP slope (65% of control). Application of MCPG (200 μM) on its own had no effect on EPSP slope. The effect of 1*S*,3*R*-ACPD was partially antagonised by MCPG (200 μM). (C) Averaged data for the effect of 1*S*,3*R*-ACPD and MCPG on paired pulse depression at interstimulus intervals of 10 and 100 ms. 1*S*,3*R*-ACPD significantly attenuated paired pulse depression at early (10 ms) and intermediate phase (100 ms) interstimulus intervals (diagonally striped bars). Following washout of ACPD, paired pulse depression returned to control values ($n = 5$; grey bars). MCPG (200 μM) had no effect on its own as seen in Fig. 5, but significantly inhibited the response to 1*S*,3*R*-ACPD at all interstimulus intervals (stippled bars). α-Methyl-L-AP4 did not block the inhibitory effect of 1*S*,3*R*-ACPD at either 10 or 100 ms inter stimulus interval (vertically striped bars; $^{*}P < 0.05$; $n = 5$; Student's *t*-test). Each point is the mean ± S.E.M.

paired responses were also compared before and after drug intervention at similar *S*1 amplitudes with no significant differences observed. Following washout of ACPD, paired pulse depression returned to control values ($n = 6$). Application of the non-specific mGlu receptor antagonist, MCPG (200 μM) had no effect on its own but blocked the transient depression of *S*1 by 1*S*,3*R*-ACPD ($n = 6$; Fig. 6A). MCPG (200 μM) also inhibited the 1*S*,3*R*-ACPD-induced reduction in paired pulse depression at early and intermediate phase interstimulus intervals ($n = 6$; Fig. 6B). α-Methyl-L-AP4 at 200 μM and 500 μM (results not shown) did not antagonise the inhibitory effect of 1*S*,3*R*-ACPD on early and intermediate paired pulse depression ($n = 5$; Fig. 5B). MCPG at 200 μM and 500 μM (results not shown) did not reverse the inhibitory effect of L-AP4 on paired pulse depression at 10 and 100 ms interstimulus intervals (Fig. 6C; $n = 5$).

A summary of the effects of the above ligands on paired pulse depression at early and intermediate interstimulus interval is shown in Table 1.

4. Discussion

We have shown that the non specific mGlu receptor group I/II agonist, 1*S*,3*R*-ACPD, the group II agonists DCG-IV and L-CCG-1 and the group III agonist, L-AP4 reduce early and intermediate phase paired pulse depression in the medial perforant path but have no effect on late phase paired pulse depression. We have also shown that the non specific mGlu receptor antagonist, MCPG, fully reverses the effects of 1*S*,3*R*-ACPD on paired pulse depression at all interstimulus intervals, the group II antagonist MCCG partially reverses the effects of DCG-IV on paired pulse depression at all interstimulus intervals and that the putative group III antagonists, α-methyl-L-AP4 and MSOP, antagonise the actions of L-AP4 at intermediate but not early phase paired pulse depression. The mGlu group I receptor agonist, (*S*)-DHPG caused a small but significant inhibition of low frequency transmission but had no effect on paired pulse depression.

Paired pulse depression is a form of synaptic plasticity that is probably mediated pre-synaptically. It is thought to result from a reduction in available transmitter caused by a reduction in pre-synaptic calcium entry (McNaughton, 1980). Such a reduction in Ca^{2+} entry, or a modulation of paired pulse depression, could be achieved by modulation of either K^{+} or Ca^{2+} currents. Kahle and Cotman (1993b) describe a transient after-hyperpolarizing K^{+} current with a decay time of about 270 ms that may be inhibited by mGlu receptors, which could explain why late phase paired pulse depression was not altered by any of the agents tested in this study. However, it has more recently been shown that, in the brainstem, mGlu receptors do not alter K^{+} currents (Takahashi et al., 1996). An alternative explanation for paired pulse depression modulation would be through the suppression of Ca^{2+} currents. In support of this theory, Takahashi et al. (1996) have shown that mGlu receptor agonists in the brainstem inhibit P/Q-type Ca^{2+} channels, while McNaughton (1980) has shown that reduction of extracellular Ca^{2+} in the medial perforant path causes a switch from the normal pattern of paired pulse depression to one of paired pulse facilitation. In addition,

we have observed that increasing the concentration of extracellular Ca^{2+} produces an even greater paired pulse depression at all interstimulus intervals (see Fig. 1).

Our findings that (*S*)-DHPG, a selective agonist of $mGlu_1$ receptor and $mGlu_5$ receptor, has no effect on paired pulse depression at all interstimulus intervals in the medial perforant path are consistent with those of Brown and Reymann (1995). Our observation of a small reduction in baseline EPSP slope may be supported by recent evidence that group I mGlu receptors mediate inhibition of synaptic transmission in the CA1 (Gereau and Conn, 1995). The non-specific group I antagonist, 4-CPG, which has been reported to be a weak antagonist at $mGlu_5$ receptor (Brabet et al., 1995; Kingston et al., 1995), when applied at high concentrations, reduced baseline EPSP slope to a greater extent than (*S*)-DHPG. This may be accounted for by the partial agonist activity of this agent at group II mGlu receptors (Hayashi et al., 1994) or more specifically $mGlu_2$ receptor (Cavanni et al., 1994), a property that might explain the effect observed when this compound is applied alone.

The non-specific mGlu receptor group II agonist L-CCG-1, when applied at 20 μm, had no effect on low frequency transmission. However, higher concentrations (50 μM) significantly reduced EPSP slope (25% of control; Fig. 2A). At both concentrations L-CCG-1 significantly reduced paired pulse depression at both early and intermediate phases. L-CCG-1 at high concentrations does have some activity at group I mGlu receptors (Nakagawa et al., 1990) and especially $mGlu_1$ receptor (Flor et al., 1996). Also it is now known that L-CCG-1 has some affinity for the $mGlu_{4a}$ receptor (Eriksen and Thompsen, 1996) and $mGlu_8$ receptor (Saugstad et al., 1997). The EC_{50} of L-CCG-I for $mGlu_2$ and $mGlu_8$ receptors are almost identical and so it is not possible to suggest whether it is acting at group II receptors alone. It has been suggested that group II mGlu receptors serve as pre-synaptic autoreceptors in the medial perforant path (Macek et al., 1996) so it is possible that it is through these autoreceptors that L-CCG-1 modulates paired pulse depression. An interesting observation in these experiments was the inhibitory effect of the putative type II antagonist (*RS*)-α-methylserine-*O*-phosphate monophenyl ester. We found that this antagonist had a marked inhibitory effect on EPSP slope and did not antagonise the effect of L-CCG-1 on paired pulse depression. We did not carry out a concentration–response effect for (*RS*)-α-methylserine-*O*-phosphate monophenyl ester and so are unable to say whether it was acting at other subtypes of mGlu receptors.

Due to the non-specificity of L-CCG-1 for group II mGlu receptors, we also examined the effects of the more specific agonist DCG-IV. DCG-IV had a marked inhibitory effect on paired pulse depression at both 10 and 100 ms interstimulus interval. In its presence a large paired pulse facilitation was observed at 100 ms interstimulus interval. The effects of DCG-IV were only partially antagonised by MCCG-1. This agent has recently been shown to be a potent and competitive antagonist at the cloned $mGlu_2$ receptor (Gomeza et al., 1996). Activity at other mGlu receptor subtypes has not yet been determined. Group II mGlu receptors comprise $mGlu_2$ receptor and $mGlu_3$ receptor, and while the $mGlu_2$ receptor is thought to be localised in the molecular layer of the dentate gyrus (Neki et al., 1996), until more specific agonists and antagonists are developed, it is not possible to determine which subtype is involved.

The non-selective group I/II mGlu receptor agonist 1*S*,3*R*-ACPD, produced a significant depressant effect on baseline EPSP slope that was reversible on addition of MCPG. The reduction in paired pulse depression observed at early and intermediate phase interstimulus intervals was also fully reversible by addition of MCPG to the perfusion medium. 1*S*,3*R*-ACPD is an mGlu receptor agonist known to enhance long term synaptic efficiency in the dentate granule cells by a post-synaptic action (Bortolotto and Collingridge, 1993; O'Connor et al., 1994). However, 1*S*,3*R*-ACPD is also known to act via pre-synaptic receptors (Burke and Hablitz, 1994; McBain et al., 1994). Given that in our studies, group I receptors seem to play no role in paired pulse depression modulation, it is likely that the 1*S*,3*R*-ACPD effect is mediated through group II mGlu receptors. However, Pin and Duvoisin (1995) have suggested that the pre-synaptic effect of 1*S*,3*R*-ACPD is mediated through the $mGlu_1$ receptor and $mGlu_5$ receptor subtypes, so an action via group I mGlu receptors cannot be ruled out.

The group III mGlu receptor agonist L-AP4 was found to depress baseline EPSP slope and reduce paired pulse depression at early and intermediate interstimulus intervals. The reduction in low frequency transmission at such a low concentration (20 μM) in the medial perforant path is unusual (Koerner and Cotman, 1981; Kahle and Cotman, 1993b). However, the reduction was slight when compared with that induced by the same concentration in the lateral perforant path (20% of control in our studies, unpublished) and it has been reported that L-AP4 at slightly higher concentrations can reduce medial perforant path EPSPs (Koerner and Cotman, 1981). It should also be noted that the inhibitory effect of L-AP4 on paired pulse depression was independent of the size of *S*1 amplitude since responses were compared at similar *S*1 amplitudes before and after drug intervention and no significant differences were observed.

L-AP4 is known to be an agonist acting pre-synaptically but independently of 1*S*,3*R*-ACPD or 1*S*,3*S*-ACPD activity (Birse et al., 1993; Jane et al., 1994; Salt and Eaton, 1995), probably via pre-synaptic group III autoreceptors. The specific group III mGlu receptor subtype which serves as an autoreceptor is not known. The group III mGlu receptor subtypes are $mGlu_{4,6,7,\text{ and }8}$ receptor. Of these, $mGlu_6$ receptor may be eliminated as it is thought to be localised exclusively in the outer zone of the inner molecu-

lar layer of the retina (Knopfel et al., 1995b). Although $mGlu_4$ receptor has the highest rank order of affinity for L-AP4 (Johansen et al., 1995) and L-AP4 is effective in activating the $mGlu_8$ receptor (Duvoisin et al., 1995), there is intense immunoreactivity with $mGlu_7$ receptor-specific antibodies on pre-synaptic terminals in the medial perforant path (Bradley et al., 1996). To add to this, it has been suggested that modulation of P-type Ca^{2+} channels might be the underlying mechanism for $mGlu_7$ receptor-mediated activity (Knopfel et al., 1995b). Therefore it is possible that the L-AP4 effects, on paired pulse depression at least, might be mediated through $mGlu_7$ receptor.

The reduction in intermediate phase paired pulse depression was completely blocked by α-methyl-L-AP4 and MSOP. MSOP is a selective group III antagonist while α-methyl-L-AP4 is thought to be a pre-synaptic mGlu receptor antagonist and thus have relative selectivity at L-AP4 sensitive receptors (Watkins and Collingridge, 1994; Pin and Duvoisin, 1995; Thoreson et al., 1995; Thoreson and Ulphani, 1995; Salt and Eaton, 1995). α-Methyl-L-AP4 has recently been shown to selectively antagonise the actions of L-AP4 in the spinal cord (Jane et al., 1994) and the lateral perforant path of the dentate gyrus (Bushell et al., 1995). More recently it has been shown that α-methyl-L-AP4 has some affinity for cloned $mGlu_2$ receptor (Gomeza et al., 1996). However, in our studies α-methyl-L-AP4 did not antagonise the effects of the group I/II agonist 1*S*,3*R*-ACPD and so in our experiments we believe α-methyl-L-AP4 acts at group III mGlu receptors only. It is likely that MSOP acts similarly to α-methyl-L-AP4 in our experiments, both having little antagonistic effect on the L-AP4 reduction in early phase paired pulse depression. α-Methyl-L-AP4, apart from acting as an apparently pure antagonist at the pre-synaptic inhibitory group III mGlu receptor, has been found to have partial agonist-like properties when measured at the cloned $mGlu_{4a}$ receptor (Johansen et al., 1995; Knopfel et al., 1995a).

The fact that α-methyl-L-AP4 and MSOP affect only intermediate phase and not early phase paired pulse depression, may indicate the existence of another subtype of receptor. It has recently been reported that a specific mGlu receptor can facilitate glutamate release (Herrero et al., 1992: Vasquez et al., 1994) which is MCPG and L-AP4 sensitive. It is linked to a PLC and not adenylate cyclase. L-AP4 acts on this receptor as an antagonist. This receptor is also found to desensitise very quickly (Herrero et al., 1994) and its developmental profile of expression changes from inhibition to facilitation with age (Vasquez et al., 1994). This desensitisation may have some role to play with responses observed within 20 ms and not those within 200 ms. This receptor may therefore be important for early phase synaptic plasticity processes.

In conclusion, we have shown that early and intermediate phase paired pulse depression is modulated by group II and III mGlu receptors. As expected, the effects produced by the group II agonists cannot be antagonised by the group III antagonist, and vice versa. It has been recently suggested that cellular responses with mixed group II/III activity may be in some cases a reflection of $mGlu_8$ receptor activity rather than additive effects of two mGlu receptors (Saugstad et al., 1997). However, until more subtype-specific mGlu receptor agonists and antagonists become available the actual subtypes involved remains unresolved.

Acknowledgements

This work was supported by grants from Forbairt (Ireland) to JJOC and DMOL. We would also like to thank University College Dublin for financial support.

References

Aramori, I., Nakanishi, S., 1992. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, $mGluR_1$, in transfected CHO cells. Neuron 8, 757–765.

Birse, E.F., Eaton, S.A., Jane, D.E., Jones, P.L.St.J., Porter, R.H.P., Pook, P.C.-K., Sunter, D.C., Udvarhelyi, P.M., Wharton, B., Roberts, P.J., Salt, T.E., Watkins, J.C., 1993. Phenylglycine derivatives as new pharmacological tools for investigating the role of metabotropic glutamate receptors in the central nervous system. Neuroscience 52, 481–488.

Bliss, T.V.P., Lomo, T., 1973. Long lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. J. Physiol. 232, 297–298.

Bortolotto, Z.A., Collingridge, G.L., 1993. Characterization of LTP induced by activation of glutamate metabotropic receptors in CA1 of the hippocampus. Neuropharmacology 32, 1–9.

Boss, V., Conn, P.J., 1992. Metabotropic excitatory amino acid receptor activation stimulates phospholipase D in hippocampal slices. J. Neurochem. 59, 2340–2343.

Brabet, I., Mary, S., Bockaert, J., Pin, J.-P., 1995. Phenylglycine derivatives discriminate between $mGluR_1$- and $mGluR_5$-mediated responses. Neuropharmacology 34, 895–903.

Bradley, S.R., Levey, A.I., Conn, P.J., 1996. Immunocytochemical localization of group III metabotropic glutamate receptors in the hippocampus with subtype specific antibodies. J. Neurosci. 16, 2044–2056.

Brown, R.E., Reymann, K.G., 1995. Metabotropic glutamate receptor agonists reduce paired-pulse depression in the dentate gyrus of the rat in vitro. Neuropharmacology 196, 17–20.

Burke, J.P., Hablitz, J.J., 1994. Presynaptic depression of synaptic transmission mediated by activation of metabotropic glutamate receptors in rat neocortex. J. Neurosci. 14, 5120–5130.

Bushell, T.J., Jane, D.E., Tse, H.-W., Watkins, J.C., Davies, C.H., Garthwaite, J., Collingridge, G.L., 1995. Antagonism of the synaptic depressant actions of L-AP4 in the lateral perforant path by MAP-4. Neuropharmacology 34, 239–241.

Cassidy, E., O'Connor, J.J., 1995. Effects of metabotropic glutamate receptors on paired pulse depression in the rat dentate gyrus in vitro. Br. J. Pharmacol. 116, 106P.

Cavanni, P., Pinnola, V., Mugaini, M., Trist, D., VanAmsredam, F.F., Feragutti, F., 1994. Pharmacological analysis of carboxyphenylglycines at metabotropic glutamate receptors. Eur. J. Pharmacol. 269, 9–15.

Conn, P.J., Boss, V., Chung, D.S., 1994. Second messengers systems coupled to metabotropic glutamate receptors. In: Conn, P.J., Patel, J. (Eds.), The Metabotropic Glutamate Receptor. Humana Press, Ottowa, pp. 59–98.

DiScenna, P.G., Teyler, T.J., 1994. Development of inhibitory and excitatory synaptic transmission in the rat dentate gyrus. Hippocampus 4, 569–576.

Duvoisin, R.M., Zhang, C., Ramonell, K., 1995. A novel metabotropic glutamate receptor expressed in the retina and olfactory bulb. J. Neurosci. 15, 3075–3083.

Eriksen, L., Thompsen, C., 1996. [H]-L-2-amino-4-phosphonobutyrate labels a metabotropic glutamate receptor $mGluR_{4\alpha}$. Br. J. Pharmacol. 116, 3279–3287.

Flor, P.J., Gomeza, J., Tones, M.A., Kuhn, R., Pin, J.-P., Knopfel, T., 1996. The C-terminal domain of the $mGluR_1$ metabotropic glutamate receptor affects sensitivity to agonists. J. Neurochem. 67, 58–63.

Gereau, R.W., Conn, P.J., 1995. Multiple pre-synaptic metabotropic glutamate receptors modulate excitatory and inhibitory synaptic transmission in hippocampal area CA1. J. Neurosci. 15, 6879–6889.

Gomeza, J., Mary, S., Brabet, I., Parmentier, M.L., Restituito, S., Bockaert, J., Pin, J.-P., 1996. Coupling of metabotropic glutamate receptors 2 and 4 to $G_{\alpha 15}$, $G_{\alpha 16}$, and chimeric $G_{\alpha q/i}$ proteins: Characterisation of new antagonists. Mol. Pharmacol. 50, 923–930.

Harris, E.W., Cotman, C.W., 1985. Effects of synaptic antagonists on perforant path paired-pulse plasticity: Differentiation of pre- and post-synaptic antagonism. Brain Res. 334, 348–353.

Hayashi, Y., Sekiyama, N., Nakanishi, S., Jane, D.E., Sunter, D.C., Birse, E.F., Udvarhelyi, P.M., Watkins, J.C., 1994. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. J. Neurosci. 14, 3370–3377.

Herrero, I., Miras-Portugal, M.T., Sanchez-Prieto, J., 1992. Positive feedback of glutamate exocytosis by metabotropic presynaptic receptor stimulation. Nature 360, 163–166.

Herrero, I., Miras-Portugal, M.T., Sanchez-Prieto, J., 1994. Rapid densensitization of the metabotropic glutamate receptor that facilitates glutamate release in rat cerebrocortical nerve terminals. Eur. J. Neurosci. 6, 115–120.

Jane, D.E., Jones, P.L.St.J., Pook, P.C.-K., Tse, H.-W., Watkins, J.C., 1994. Actions of two new antagonists showing selectivity for different subtypes of metabotropic glutamate receptor in the neonatal rat spinal cord. Br. J. Pharmacol. 112, 809–816.

Johansen, P.A., Chase, L.A., Sinor, A.D., Koerner, J.F., Johnson, R.L., Robinson, M.B., 1995. Type 4α metabotropic glutamate receptor: Identification of new potent agonists and differentiation from the L-(+)-2-amino-4-phosphonobutanoic acid-sensitive receptor in the lateral perforant pathway in rats. Mol. Pharmacol. 48, 140–149.

Kahle, J.S., Cotman, C.W., 1993a. Adenosine, L-AP4 and baclofen modulation of paired pulse potentiation in the dentate gyrus: Interstimulus interval-dependent pharmacology. Exp. Brain Res. 94, 97–104.

Kahle, J.S., Cotman, C.W., 1993b. L-2-amino-4-phosphonobutanoic acid and 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid reduce paired pulse depression recorded from the medial perforant path in the dentate gyrus of rat hippocampal slices. J. Pharmacol. Exp. Ther. 266, 207–215.

Kingston, A.E., Burnett, J.P., Mayne, N.G., Lodge, D., 1995. Pharmacological analysis of 4-carboxyphenylglycine derivatives: Comparison of effects on $mGluR_{1\alpha}$ and $mGluR_{5a}$ subtypes. Neuropharmacology 34, 887–894.

Koerner, J.F., Cotman, C.W., 1981. Micromolar L-2-amino-4-phosphonobutyric acid selectively inhibits perforant path synapses from lateral entorhinal cortex. Brain Res. 216, 192–198.

Knopfel, T.S., Kuhn, R., Allegier, H., 1995a. Metabotropic glutamate receptor: Novel targets for drug developement. J. Med. Chem. 38, 1417–1426.

Knopfel, T.S., Lukic, T., Leonardt, P.J., Flor, R., Kuhn, R., Gasparini, F., 1995b. Pharmacological characterisation of MCCG and MAP-4 at the $mGluR_{1b}$, $mGluR_2$ and $mGluR_{4a}$ human metabotropic glutamate receptor subtypes. Neuropharmacology 34, 1099–1102.

McBain, C.J., DiChiara, T.J., Kauer, J.A., 1994. Activation of metabotropic glutamate receptors differentially affects two classes of hippocampal interneurons and potentiates excitatory synaptic transmission. J. Neurosci. 14, 4433–4445.

McNaughton, B.L., 1980. Evidence for 2 physiologically distinct perforant pathways to the fascia dentata. Brain Res. 199, 1–19.

Macek, T.A., Winder, D.G., Gereau, R.W., Ladd, C.O., Conn, J.A., 1996. Differential involvement of group II and III mGluRs as autoreceptors at lateral and medial perforant path synapses. J. Neurophysiol. 76, 3798–3806.

Nakagawa, Y., Saitah, K., Ishihara, T., Ishida, M., Shinozaki, H., 1990. (2*S*,3*S*,4*S*)α-(Carboxycyclopropyl) glycine is a novel agonist of metabotropic glutamate receptors. Eur. J. Pharmacol. 184, 205–206.

Neki, A., Ohishi, H., Kaneko, T., Shigemoto, R., Nakanishi, S., Mizuno, N., 1996. Pre and postsynaptic localization of a metabotropic glutamate receptor, $mGluR_2$, in the rat brain: An immunohistochemical study with a monoclonal antibody. Neurosci. Lett. 202, 197–200.

O'Connor, J.J., Rowan, M.J., Anwyl, R., 1994. Long-lasting enhancement of NMDA-receptor mediated synaptic transmission by metabotropic glutamate receptor activation. Nature 367, 557–559.

Okamoto, N., Hore, S., Akazawa, C., Hayashi, Y., Shigemoto, R., Mizuno, N., Nakanishi, S., 1994. Molecular characterization of a new metabotropic glutamate receptor $mGluR_7$ coupled to inhibitory cyclic AMP signal transduction. J. Biol. Chem. 269, 1231–1236.

O'Leary, D.M., O'Connor, J.J., 1997. Effects of type I and II metabotropic glutamate receptor agonists and antagonists on paired pulse depression in the rat dentate gyrus in vitro. Br. J. Pharmacol. 120, 31.

Pellegrini-Giampietro, D.E., Torregrossa, S.A., Moroni, F., 1996. Pharmacological characterisation of metabotropic glutamate receptors coupled to phospholipase D in the rat hippocampus. Br. J. Pharmacol. 118, 1035–1043.

Pin, J.-P., Duvoisin, R., 1995. The metabotropic glutamate receptors: Structure and functions. Neuropharmacology 34, 1–26.

Salt, T.E., Eaton, S.A., 1995. Distinct presynaptic metabotropic receptors for L-AP4 and L-CCG-1 on GABAergic terminals: Pharmacological evidence using novel α-methyl derivative mGluR antagonists, MAP-4 and MCCG, in the rat thalamus in vivo. Neuroscience 65, 5–13.

Saugstad, J.A., Kinzie, J.M., Shinohara, M.M., Segerson, T.P., Westbrook, G.L., 1997. Cloning and expression of rat $mGluR_8$ reveals a distinct pharmacological profile. Mol. Pharmacol. 51, 119–125.

Schoepp, D.D., Bockaert, J., Sladeczek, F., 1990. Pharmacological and functional characteristics of metabotropic excitatory amino acid receptors. Trends Pharmacol. Sci. 11, 508–515.

Schoepp, D.D., Conn, P.J., 1993. Metabotropic glutamate receptors in brain function and pathology. Trends Pharmacol. Sci. 14, 13–20.

Schulz, P.E., Cook, E.P., Johnston, D., 1995. Using paired-pulse facilitation to probe the mechanisms for long-term potentiation (LTP). J. Physiol. (Paris) 89, 3–9.

Takahashi, T., Forsyth, I.D., Tsujimoto, T., Barnes-Davies, M., Onodera, K., 1996. Presynaptic calcium current modulation by metabotropic glutamate receptors. Science 274, 594–597.

Thoreson, W.B., Ulphani, J.S., 1995. Pharmacology of selective and non-selective metabotropic glutamate receptor agonists at L-AP4 receptors in retinal ON bipolar cells. Brain Res. 676, 93–102.

Thoreson, W.B., Velte, T.J., Miller, R.F., 1995. Actions of phenylglycine derivatives at L-AP4 receptors in retinal ON bipolar cells. Neuropharmacology 34, 27–34.

Trombley, P.Q., Westbrook, G.L., 1992. L-AP4 inhibits calcium currents and synaptic transmission via G-protein-coupled glutamate receptor. J. Neurosci. 12, 356–357.

Vasquez, E., Herrero, I., Miras-Portugal, M.T., Sanchez-Prieto, J., 1994. Facilitation of metabotropic glutamate receptors in hippocampal nerve terminals. Neurosci. Res. Commun. 15, 187–194.

Watkins, J., Collingridge, G.L., 1994. Phenylglycine derivatives as antagonists of metabotropic glutamate receptors. Trends Pharmacol. Sci. 15, 333–342.