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Developmental regulation of hippocampal excitatory synaptic transmission by metabotropic glutamate receptors

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1 The aims of this study were, to use agonists selective for the 3 mGlu receptor groups to identify developmental changes in their effects, and to assess the usefulness of proposed selective antagonists as pharmacological tools.

2 Hippocampal slices (400 μm) were prepared from neonate (9–14 days) and young adult (5–7 weeks) Sprague-Dawley rats. Field excitatory postsynaptic potentials (fEPSP) were recorded from CA1.

3 DHPG (100 μM), a group I agonist, produced a slowly developing enhancement of fEPSP slope in slices from adults. In slices from neonates, DHPG (75 μM) depressed fEPSP slope.

4 DCG-IV (500 nM), a group II agonist, did not affect the fEPSP recorded from slices from adults whereas perfusion in neonate slices produced a sustained depression.

5 The group III agonist *L*-AP4 (50 μM) was ineffective in adult slices but depressed fEPSP slope in slices prepared from neonates.

6 DHPG-induced depression of fEPSP slope was inhibited by 4-CPG (400 μM), a group I antagonist, but was unaffected by MCCG (500 μM) and MAP4 (500 μM), group II and III receptor antagonists respectively. MCCG but not MAP4 antagonized the effects of DCG-IV with 4-CPG producing variable effects. The effect of *L*-AP4 was unaffected by MCCG, blocked by MAP4, and enhanced by 4-CPG.

7 The results show that the effects of the agonists for all groups of mGlu receptors are developmentally regulated. Furthermore, MCCG and MAP4 behave as effective and selective antagonists for group II and group III mGlu receptors respectively, whereas the usefulness of 4-CPG as a group I antagonist may be limited.
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Abbreviations: ACPD, (1*S*, 3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; aCSF, artificial cerebrospinal fluid; AIDA, (RS)-1-aminoindan-1,5-dicarboxylic acid; 4-CPG, (*S*)-4-carboxyphenylglycine; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG, (S)-3-dihydroxyphenylglycine; fEPSP, field excitatory postsynaptic potentials; *L*-AP4, L-(+)-2-amino-4-phosphonobutyric acid; LTD, long term depression; MAP4, (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid; MCCG, (2*S*,3*S*,4*S*)-2-methyl-2(carboxycyclopropyl)glycine; mGlu, metabotropic glutamate

Introduction

There exists little dispute regarding the fact that metabotropic glutamate receptors (mGlu receptors) play an important role both in normal synaptic transmission and in synaptic plasticity (long-term potentiation and long-term depression) within the hippocampus. However numerous, often conflicting, reports exist with regard to the exact nature of mGlu receptor modulation of synaptic activity. Initial investigations centred around the use of (1*S*, 3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) a mixed group I and II mGlu receptor agonist (Palmer *et al*., 1989). Brief application of ACPD produced a reversible concentration-dependent depression in the field excitatory postsynaptic potential (fEPSP) recorded from the CA1 region of hippocampal slices (Baskys & Malenka, 1991b; Manzoni & Bockaert, 1995; Harvey *et al*., 1996). In contrast similar applications of ACPD have been shown to be ineffective (Overstreet *et al*., 1997) or to enhance the fEPSP (Bortolotto & Collingridge, 1993; Collins *et al*., 1995). Although the emergence of more selective mGlu receptor agonists has allowed greater insight into the modulation of synaptic transmission by mGlu receptors, discrepancies are still apparent. One likely reason for the array of effects noted on application of mGlu receptor agonists could be the different age of the animals. The aim of this study was to use agonists selective for the three mGlu receptor groups to identify developmental changes in their effects on low frequency evoked synaptic transmission in hippocampal slices, and to assess the usefulness of proposed selective antagonists as pharmacological tools.

Methods

Halothane anaesthetized female Sprague-Dawley rats (5–7 weeks old) were given a blow to the thorax prior to being decapitated. Neonatal Sprague-Dawley rats (9–14 days old) of either sex were decapitated whilst under isofluothane induced anaesthesia. Transverse hippocampal slices (400 μm) were cut using a McIlwain tissue chopper and incubated in artificial cerebrospinal fluid (aCSF) for at least 1 h before use in a gassed (95%O_2-5%CO_2) holding chamber. The aCSF contained (in mM): NaCl 124, KCl 3, $NaHCO_3$ 26, NaH_2PO_4

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1.25, D-glucose 10, $MgSO_4$ 1 and $CaCl_2$ 2, saturated with 95%O_2-5%CO_2. The CA3 region was removed with a scalpel prior to the slices being transferred to an interface chamber continually perfused with (aCSF) at a constant 28–30°C.

A bipolar stimulating electrode was placed in the stratum radiatum of the CA1 region to allow orthodromic stimulation of the Schaffer-collateral commissural fibres. Field excitatory postsynaptic potentials (fEPSP) were recorded from the CA1 dendritic layer using borosilicate glass capillary electrodes (resistance 2–9 MΩ) filled with 3 M NaCl. Stimulation was applied at 0.033 Hz and stimulus intensity was set to evoke a fEPSP of approximately half-maximal amplitude. Drugs were applied by addition to the perfusion medium.

fEPSP slope was constantly measured using the LTP data program (Anderson & Collingridge, 1997) www.ltp-program.com. The control fEPSP slope was calculated as the mean of the responses 15 min immediately before drug addition. The drug effect elicited was calculated at the point of maximal effect. Results are expressed as percentage of control ± standard error of the mean (s.e.mean). Agonists were perfused for a 5 min period with a minimum of 30 min between subsequent additions. Antagonists were perfused for a period of 5 min prior to and during perfusion of agonists. Statistical analysis was performed on raw data using a paired Student's *t*-test. $P<0.05$ was taken to indicate significance. In all figures a black bar indicates drug application.

Materials

Stock solutions of (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA), (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV), L-(+)-2-amino-4-phosphonobutyric acid (*L*-AP4), (*S*)-4-carboxyphenylglycine (4-CPG), (2*S*,3*S*,4*S*)-2-methyl-2-(carboxycyclopropyl)glycine (MCCG) and (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4) were all prepared using equimolar NaOH. (S)-3-dihydroxyphenylglycine (DHPG) was dissolved in distilled water to form a stock solution. Stock solutions were diluted in aCSF prior to perfusion. All drugs were obtained from Tocris Cookson.

Results

Effect of mGlu receptor agonists

The initial objective was to investigate the effect of a 5-min perfusion of selective mGlu receptor agonists on the slope of the fEPSP recorded from the CA1 region of hippocampal slices prepared from, (a) young adult, and (b) neonatal rats.

DHPG (100 μM), a selective group I agonist (Schoepp *et al*., 1994) did not have an immediate effect on fEPSP slope in slices from young adult rats (103±6% control at 4–8 min after start of drug perfusion, $n=6$) (Figure 1a). In some slices a slowly developing potentiation was produced, however, this was inconsistent and the trend which is evident in Figure 1a (187±38% of control at 60 min) was not statistically significant in the whole sample. In slices from neonates, a comparable concentration of DHPG (75 μM) caused a reversible depression in fEPSP slope (74±6% of control, 4–8 min after start of drug perfusion, $P<0.05$, $n=4$). No potentiation in fEPSP slope was apparent up to 30 min after DHPG had been perfused.

The selective group II agonist DCG-IV (500 nM) (Hayashi *et al*., 1993) had no effect on the fEPSP recorded from adult hippocampal slices (104±6% of control 60 min after drug perfusion, $n=4$) (Figure 2a). However, in hippocampal slices prepared from neonates, DCG-IV (500 nM) produced a depression in fEPSP slope which was sustained for at least 60 min after the start of drug perfusion (43±9% of control 60 min after DCG-IV perfusion, $P<0.05$, $n=4$) (Figure 2b).

Figure 3 illustrates the effect of perfusion of *L*-AP4, which is a selective agonist for group III mGlu receptors (Pin & Duvoisin, 1995). As with DCG-IV, a 5 min perfusion of *L*-AP4 (50 μM) produced no change in the fEPSP slope in slices from young adult rats (Figure 3a), however, in slices from neonates *L*-AP4 (50 μM) caused a reversible decrease in fEPSP slope to 55±1% of control 5–10 min after the start of drug perfusion ($P<0.05$, $n=3$) (Figure 3b).

Due to the consistent effects of the agonists in the hippocampal slices prepared from neonates, the selectivity of the agonists was investigated using 4-CPG, MCCG and MAP4 which are proposed to be selective antagonists for group I (Birse *et al*., 1993), group II (Jane *et al*., 1994) and group III (Jane *et al*., 1994) mGlu receptors respectively. In this series of experiments, in order to allow a paired comparison and eliminate inter-slice variability, agonist effects in the absence and presence of antagonist were tested in the same slices.

Antagonism of DHPG effect in slices from neonates

A control response to DHPG was obtained in all slices used with a 30-min period between the first addition of DHPG and the start of the antagonist experiments. In the absence of any antagonist DHPG (75 μM) depressed fEPSP slope to 56±5% of control ($P<0.05$, $n=4$), however, this effect was completely blocked in the presence of 4-CPG (400 μM) (fEPSP slope being 94±7% of control 5–10 min after start of DHPG perfusion, Figure 4a). This difference in effect was significant ($P<0.05$, $n=4$). In contrast, neither MCCG (500 μM), nor MAP4 (500 μM) affected the depression evoked by DHPG. The control depression produced by DHPG illustrated in Figure 4b (68±8% of control, $P<0.05$, $n=4$) was not significantly different in the presence of MCCG (500 μM) (65±5% of control, $P<0.05$, $n=4$). Figure 4c shows the effect of DHPG alone (62±8% of control, $P<0.05$, $n=5$) and when perfused with MAP4 (500 μM) (80±8% of control, $P<0.05$, $n=5$). Although the extent of depression induced by DHPG is reduced in the presence of MAP4 this does reach significance.

Although in a small number of DHPG experiments the fEPSP slope 30-min after the end of DHPG perfusion was smaller than that prior to drug addition this never reached significance and thus LTD never occurred.

Antagonism of DCG-IV effect in slices from neonates

Due to the irreversible depression that DCG-IV produces, the antagonist study was conducted first, followed after a 30-min wash period by the addition of DCG-IV alone. The results with DCG-IV and the putative group I antagonist 4-CPG (400 μM) were variable. In four out of five slices the fEPSP slope was depressed 30 min after perfusion with 4-CPG + DCG-IV but in the remaining slice no inhibition was apparent, therefore, DCG-IV in the presence of 4-CPG did not significantly alter the fEPSP slope (74±10% of control, $P>0.05$, $n=5$) (Figure 5a). Although the subsequent perfusion of DCG-IV alone produced a less apparent acute effect, fEPSP slope was still significantly decreased 30 min after DCG-IV perfusion (60±6% of control, $P<0.05$, $n=5$). There was no significant difference between the depressions evoked by DCG-IV and DCG-IV + 4-CPG ($P>0.05$, $n=5$).

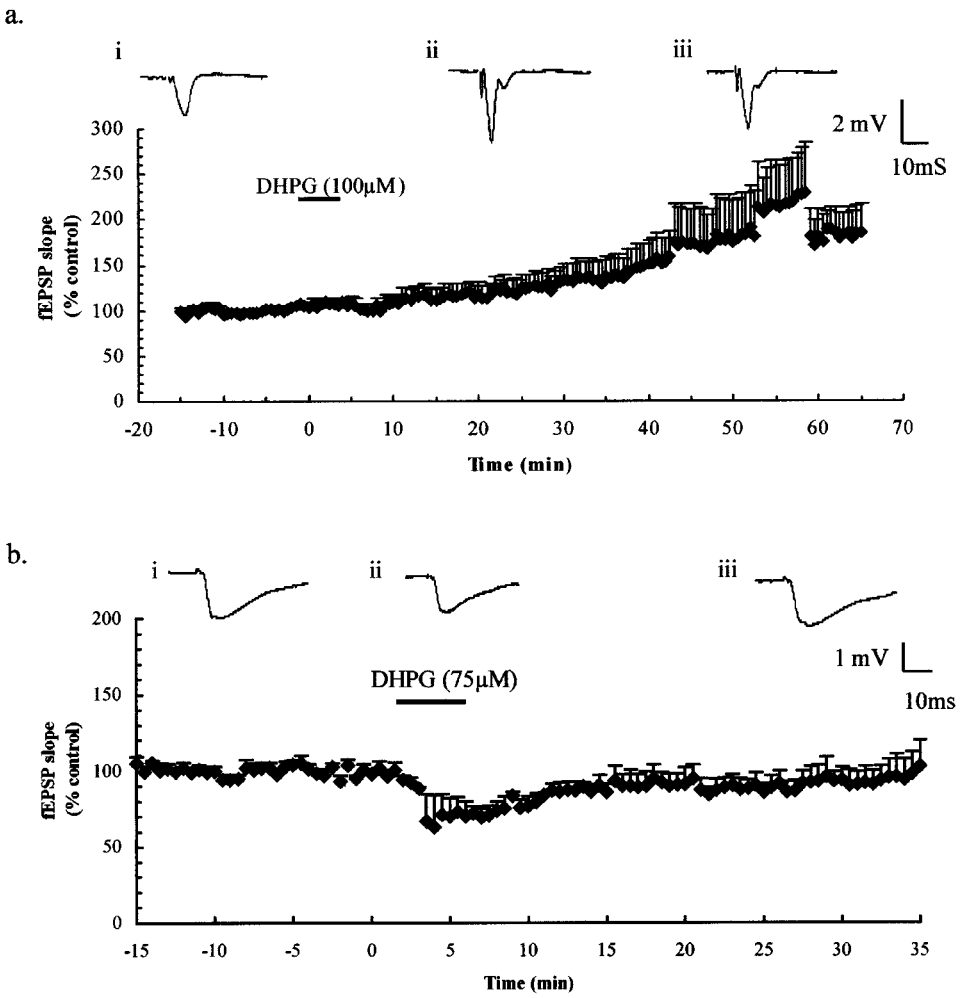

Figure 1 (a) The perfusion of DHPG, a mGlu receptor group I agonist, for 5 min did not have any immediate consistent effect on fEPSP slope however a slow onset potentiation was apparent in five out of six hippocampal slices ($187 \pm 38\%$ of control at 60 min post drug perfusion, $n=6$, $P>0.05$). Graph shows pooled data and traces are from a representative slice at i control, ii 30 min wash and iii 60 min wash. (b) In slices from neonates DHPG (75 μM) caused a reversible decrease in fEPSP slope ($74 \pm 6\%$ of control at 4–8 min after drug perfusion, $n=4$, $P<0.05$). Example traces were taken at i control, ii max effect of DHPG and iii 30 min wash.

No change in fEPSP slope occurred in the 5-min perfusion of 4-CPG prior to addition of DCG-IV. Figure 5b shows that the group II antagonist MCCG reduced both the initial and sustained depression in fEPSP slope produced by DCG-IV (1 μM) ($86 \pm 5\%$ of control against $69 \pm 7\%$ of control, $n=4$, $P<0.05$). The decrease in fEPSP slope at 30 min after perfusion of DCG-IV was not changed in the presence of MAP4 (500 μM) (Figure 5c). The fact that two consecutive perfusions of DCG-IV produce comparable depressions in fEPSP slope suggests that the LTD that follows a 5-min perfusion of DCG-IV (1 μM) is not saturated.

Antagonism of L-AP4 effect in slices from neonates

As with DHPG, control responses to *L*-AP4 were first obtained in each slice and then compared with the effect produced when *L*-AP4 was perfused in parallel with either 4-CPG, MCCG or MAP4. In Figure 6a the application of *L*-AP4 alone reduced fEPSP slope to $62 \pm 3\%$ of control ($P<0.05$, $n=3$), and in the presence of 4-CPG the fEPSP was further depressed to $27 \pm 5\%$ of control ($P<0.05$). This enhanced depression was significant at the 90% level. No such alteration in effect was seen with MCCG (Figure 6b). In this group of slices, perfusion of *L*-AP4 alone depressed fEPSP slope to $80 \pm 5\%$ of control ($P<0.05$, $n=5$), and in the presence of MCCG it was reduced to $79 \pm 4\%$ of control ($P<0.05$, $n=5$). As expected the depression of the fEPSP produced by *L*-AP4 was totally inhibited by MAP4 (Figure 6c).

Discussion

Since the discovery that glutamate can activate not only ionotropic but also metabotropic receptors (Sladeczek *et al*., 1985; Sugiyama *et al*., 1987) a great deal of work has been carried out to unravel the involvement of mGlu receptors in governing synaptic activity, with results that are sometimes contradictory. The main aims of this study were (a) to investigate if differing results obtained are in part due to the use of animals at different stages of development and (b) whether proposed antagonists for the three mGlu receptor groups are useful experimental tools.

The effect of mGlu receptor agonists in hippocampal slices from young adult rats

Group I mGlu receptors Group I mGlu receptors, which constitutes mGlu1 and 5 plus their splice variants, are

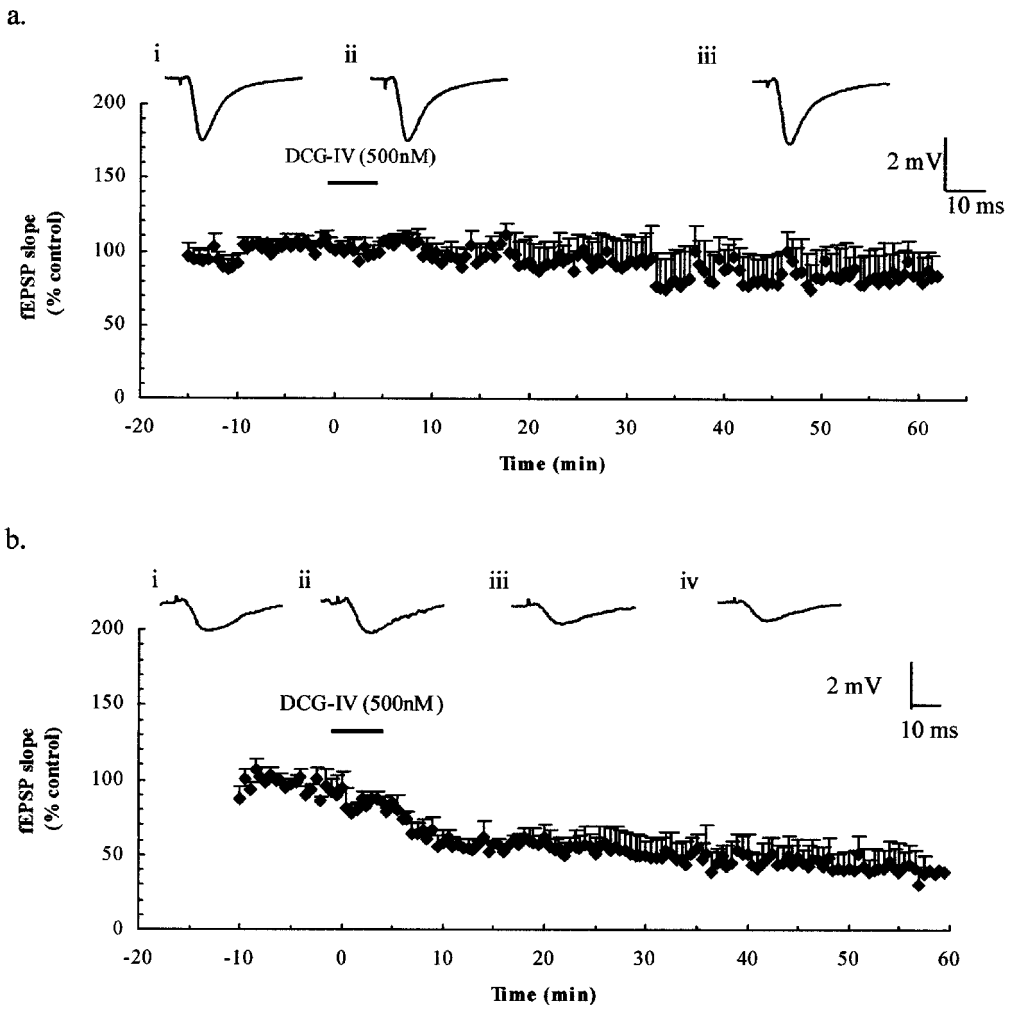

Figure 2 (a) DCG-IV, a mGlu receptor group II agonist, perfused for 5 min, had no effect on fEPSP slope in adult hippocampal slices ($104 \pm 6\%$ of control 60 min after drug perfusion, $n=4$, $P>0.05$). (b) DCG-IV caused sustained depression of the fEPSP ($43 \pm 9\%$ of control 60 min after drug perfusion, $n=4$, $P<0.05$) in hippocampal slices from neonatal rats. In (a) i–iii are examples of synaptic responses at control, end of DCG-IV perfusion and 60 min wash and in (b) i–iv are from control, end of DCG-IV perfusion and 30 and 60 min wash.

classified functionally by stimulation of phospholipase C (Masu *et al*., 1991) and pharmacologically through activation by DHPG (Schoepp *et al*., 1994). Whilst not reaching statistical significance in the whole sample, the addition of DHPG to hippocampal slices from young adult rats did produce a slowly developing potentiation in some slices. Comparable results have been reported using ACPD (10 μM), a mixed group I/II mGlu receptor agonist, which caused a slow onset, sustained potentiation of the fEPSP in the CA1 region of hippocampal slices prepared from adult rats (Bortolotto & Collingridge, 1993). However in hippocampal slices in which the CA3 region had been removed no such potentiation ensued after ACPD perfusion (Collins & Davies, 1994) leading to the suggestion that the ACPD-induced potentiation was an indirect effect resulting from an increased excitatory drive from the CA3 neurones (Collins *et al*., 1995). Since in the present study the CA3 was always removed it is possible that this is not the only mechanism of potentiation. However it has to be noted that potentiation did not occur in all slices. Therefore the probability exists that in those slices in which potentiation occurred, not all the CA3 region had been removed. This is supported by the example trace in Figure 1, which shows that the potentiation of the fEPSP is accompanied by an increase in fibre volley amplitude. Injections of ACPD into the CA1 region *in vivo* induced an enduring potentiation of fEPSP (Davis & Laroche, 1996; Manahan-Vaughan *et al*., 1999). The ability of DHPG to emulate the effect of ACPD (Davis & Laroche, 1996; Manahan-Vaughan & Reymann, 1997) together with the inhibition of ACPD induced potentiation by 4-CPG (Manahan-Vaughan *et al*., 1999) suggests that this ACPD mediated potentiation is due to activation of group I receptors. The mechanisms by which group I mGlu receptors might potentiate synaptic responses are not clear but at least three possibilities have been suggested. First, there might be a direct effect as a consequence of PLC produced second messengers (Bortolotto & Collingridge, 1993); secondly, an indirect effect *via* a group I mGlu receptor mediated enhancement of NMDA receptor function (reviewed in Ben-Ari & Aniksztejn, 1995) or third, some more complex interaction between NMDA and group I mGlu receptors controlling the release of glutamate (reviewed in Sanchez-Prieto *et al*., 1996). In contrast to the above Palmer *et al*. (1997) reported that DHPG (100 μM) perfused for 10 min results in a form of LTD in hippocampal slices prepared from young adult rats. Although this effect was apparent under normal conditions it was greatly enhanced by using a Mg^{2+}-free medium or by adding picrotoxin to increase

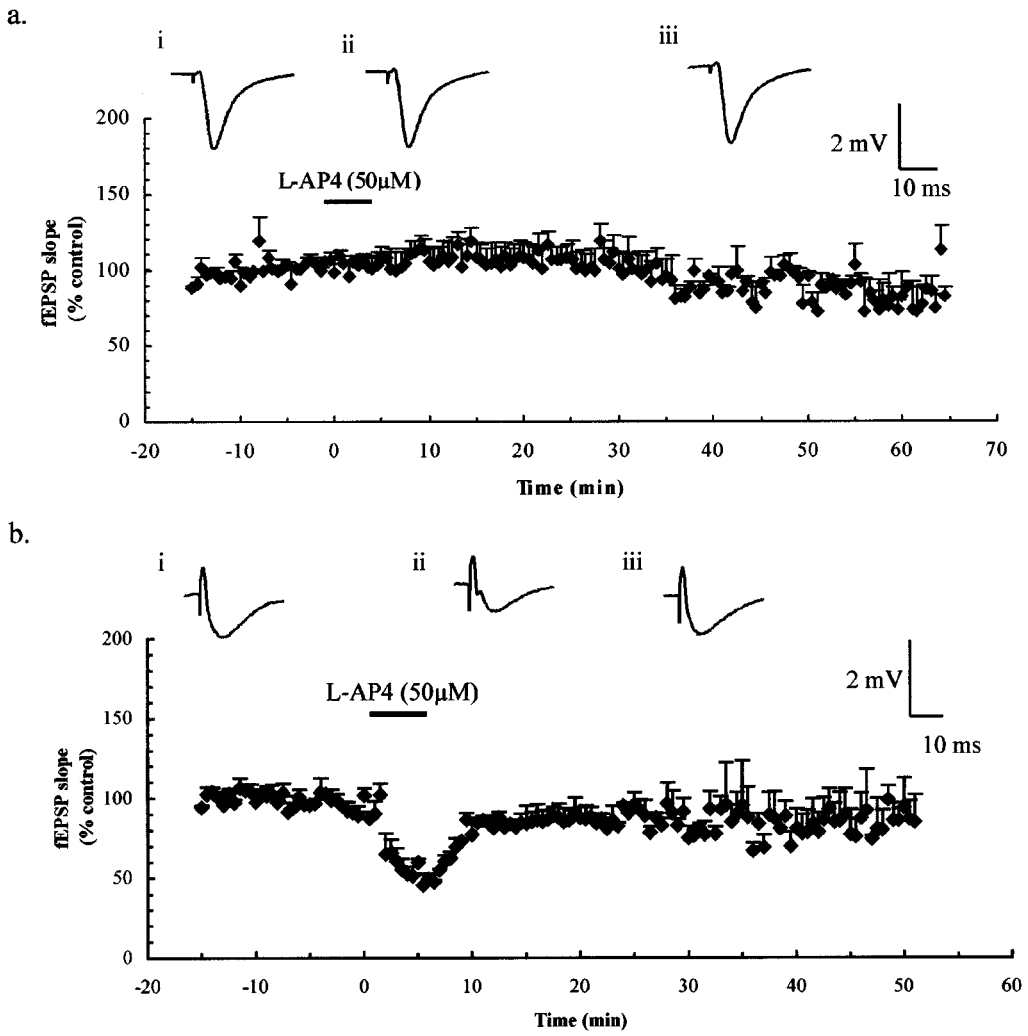

Figure 3 (a) The mGlu receptor group III agonist, *L*-AP4, perfused for 5 min, had no effect on fEPSP slope in young adult rat hippocampal slices (104±8% control 5–10 min after the start of drug perfusion, $n=5$, $P>0.05$). (b) A similar perfusion of *L*-AP4 (50 μM) in neonatal rat hippocampal slices caused a reversible depression in fEPSP slope (55±1% control 5–10 min after drug perfusion, $n=3$, $P<0.05$). In both instances the traces are from representative slices at i control, ii end of *L*-AP4 perfusion and iii 30 min wash.

excitability. DHPG-induced LTD in the dentate gyrus of hippocampal slices prepared from rats 3–5 weeks old was not dependent upon suppressing inhibition (Camodeca *et al*., 1999).

Group II mGlu receptors The subtypes mGlu2 and 3 belong to the group II mGlu receptors. The rank order of potency for agonist activation of this group is DCG-IV > = L-CCG-I > Glu > ACPD > quisqualate (Pin & Duvoisin, 1995). With regard to transduction mechanism, when expressed in mammalian cells these receptors cause a reduction in cyclic AMP formation induced by forskolin or a G_s-coupled receptor. In our experiments the perfusion of DCG-IV (500 nM) for 5 min produced no acute or chronic effect on the fEPSP amplitude. This is largely in line with previous experiments where fEPSPs evoked by stimulation of the Schaffer-collateral commissural pathway and recorded in CA1 stratum radiatum were only reduced to 92.1±1% of control by DCG-IV (0.1 μM) in slices of hippocampus from rats 20–30 days old (Kamiya *et al*., 1996). DCG-IV (100 nM) and L-CCG-I also failed to affect synaptic responses recorded in a Mg^{2+}-free medium from the CA1 region of hippocampal slices of 4–10 week old rats (Harvey *et al*., 1996), and a recently developed selective group II receptor agonist LY354740 (Schoepp *et al*., 1997; Monn *et al*., 1997) had no obvious effect on fEPSP amplitude in CA1 (Kilbride *et al*., 1998).

Group III mGlu receptors Group III mGlu receptors are comprised of mGlu4, 6, 7 and 8. As with group II receptors, these are negatively linked to adenylyl cyclase *via* PTX-sensitive G-proteins when expressed in Chinese hamster ovary (CHO) or baby hamster kidney (BHK) cells (Conn & Pinn, 1997). Pharmacologically, group III receptors are characterized by being selectively activated by *L*-AP4 and L-serine-O-phosphate (L-SOP), however, *L*-AP4 has a very low potency at mGlu7. The lack of effect of *L*-AP4 in slices prepared from young adult rats (35–49 days old) mirrors previous results where the application of *L*-AP4 (50 μM) to slices of hippocampus prepared from 80–150 day old rats had no effect on synaptic transmission in the CA1 region (Baskys & Malenka, 1991a). Under conditions of zero-Mg^{2+} aCSF *L*-AP4 (10 μM) also had little effect on the fEPSP slope recorded in the CA1 region of slices from 4–10 week old rats (Harvey *et al*., 1996). In contrast, in a modified medium containing picrotoxin, *L*-AP4 was reported to cause a dose-dependent reversible depression of the EPSP recorded in the CA1 region of adult hippocampal slices (Manzoni & Bockaert, 1995). The reason for this is not clear.

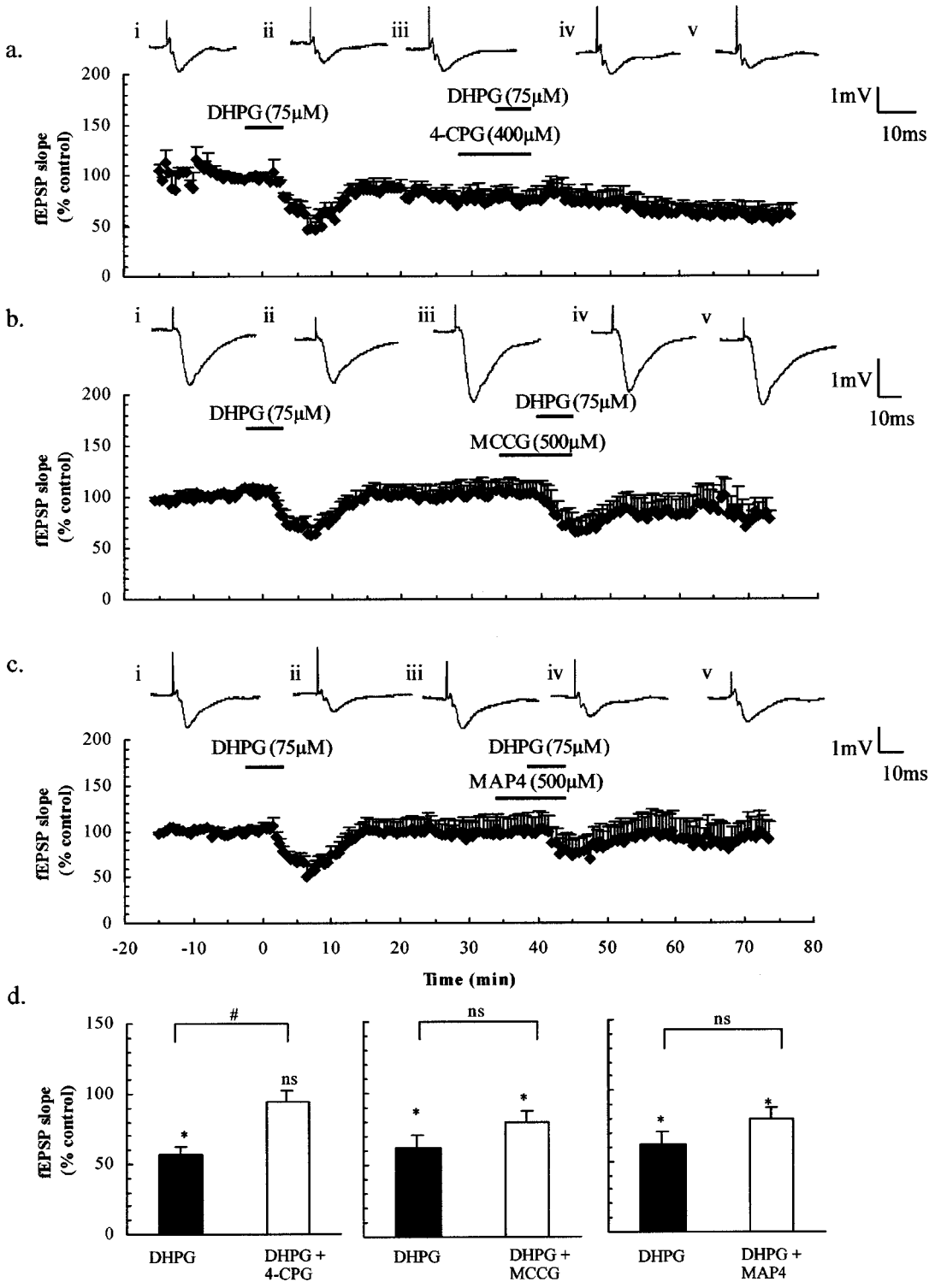

Figure 4 4-CPG, but not MCCG or MAP4, blocked the effect of DHPG in slices prepared from neonate rats. In all cases DHPG alone depressed fEPSP amplitude significantly. (a) The group I antagonist 4-CPG (400 μM) significantly inhibited this depression. In the presence of (b) MCCG (500 μM) and (c) MAP4 (500 μM), antagonists for group II and III mGlu receptors respectively, DHPG still significantly depressed the fEPSP. (d) Shows a summary of the results. In all figures traces are from representative slices at i control, ii maximum effect of DHPG, iii 30 min after DHPG perfusion, iv effect of DHPG in presence of antagonist and v after 30 min wash.

The effect of mGlu receptor agonists and antagonists in hippocampal slices from neonates

Group I mGlu receptors In complete contrast to the situation in slices from adults, the application of DHPG (75 μM) to slices of hippocampus from neonates (9–14 days old) resulted in a brief reversible depression of fEPSP slope. This is not due to the difference in concentration used as DHPG (100 μM) also decreased the fEPSP (data not shown). Previous experiments have reported that trans-ACPD (50 μM) had a minimal effect on synaptic transmission in adult slices but depressed EPSP slope by approximately 50% in slices from rats aged 11–25 days old (Baskys & Malenka, 1991a). Similarly, Overstreet *et al.* (1997) found (1*S*,3*R*)-

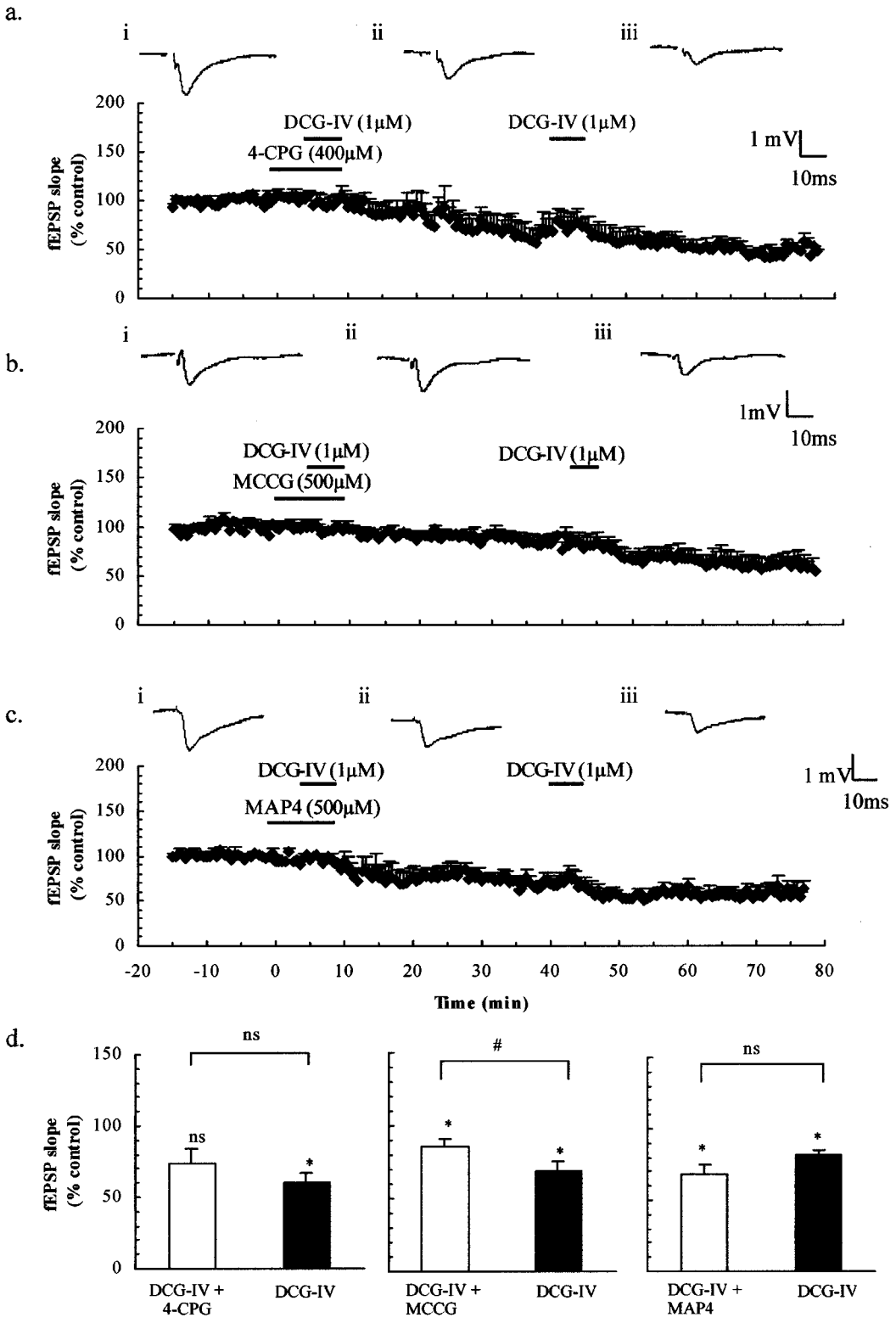

Figure 5 MCCG, but not MAP4 blocked the effect of DCG-IV in slices prepared from neonate rats. 4-CPG had variable effects. (a) In the presence of the group I antagonist 4-CPG (400 μM), DCG-IV did not significantly depress the fEPSP. However, it is important to note that this was due to a large variation in response in the presence of 4-CPG, rather than to consistent blockage of the effects of DCG-IV, hence there was no significant difference between DCG-IV and DCG-IV + 4-CPG. (b) The group II antagonist MCCG (500 μM) inhibited the sustained depression produced by DCG-IV (1 μM) whilst the group III antagonist MAP4 (500 μM) did not alter the effect of DCG-IV (c). Results are summarized in (d). Synaptic responses are from representative slices at i control, ii 30 min after end of DCG-IV + antagonist perfusion and iii 30 min after end of DCG-IV perfusion.

ACPD (10 μM) to have a minimal or excitatory effect in slices from 28–33 day old rats but to cause a rapid and long lasting depression of the EPSP slope in 8–12 day old rat slices. These effects could, however, be explained by the action of ACPD on group II mGlu receptors (see below). Experiments using the selective group I agonist DHPG (30 μM) have reported either a brief or long lasting depression of the fEPSP recorded from the CA1 region of

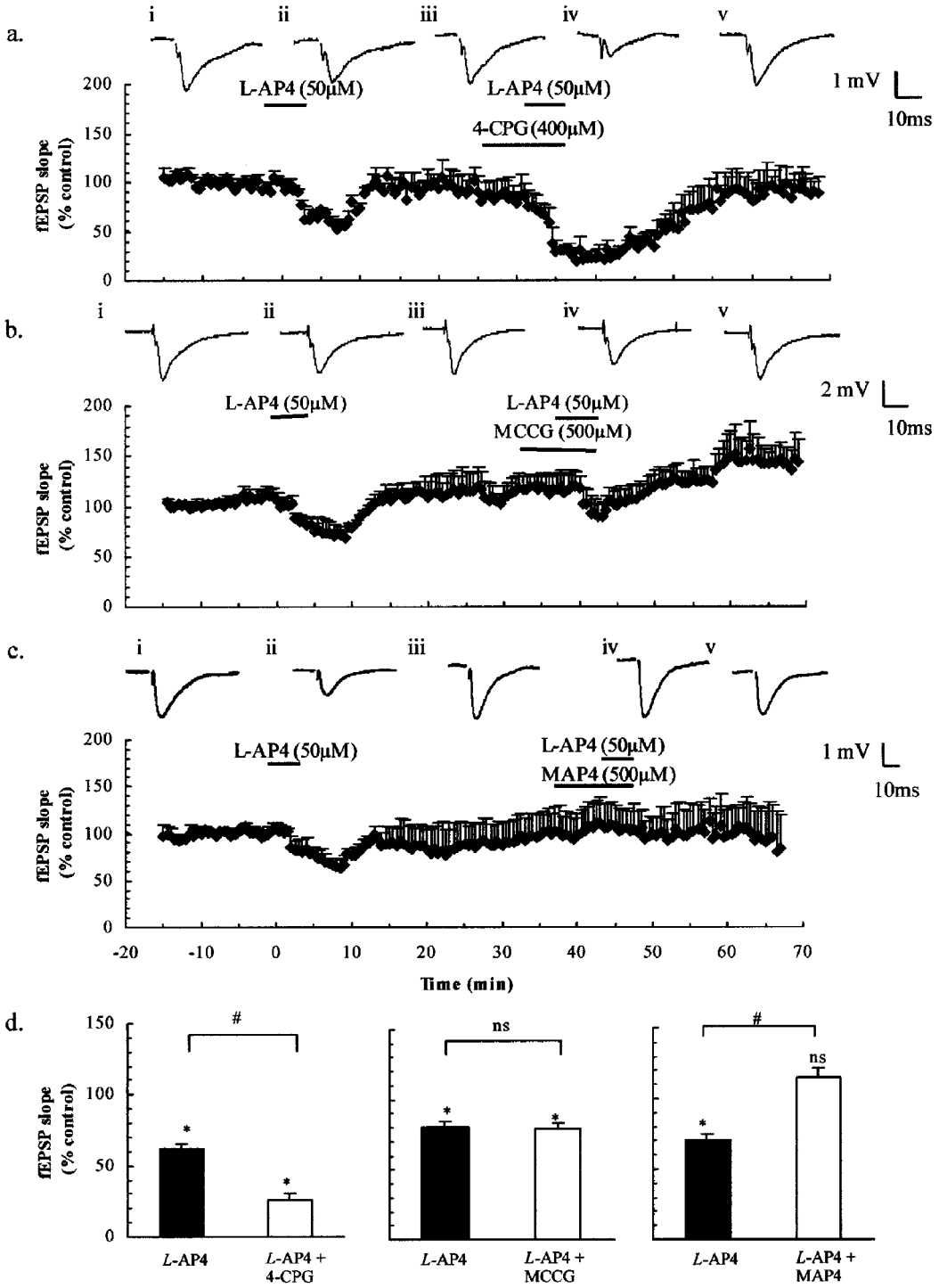

Figure 6 MAP4, but not MCCG blocked the effect of *L*-AP4 in slices prepared from neonate rats. 4-CPG potentiated the effect of *L*-AP4. (a) The perfusion of *L*-AP4 and 4-CPG produced a reversible depression of fEPSP slope that was greater than that by *L*-AP4 alone. (b) The effect of *L*-AP4 was not altered by the presence of MCCG, a group II mGlu receptor antagonist. (c) In contrast to this MAP4 totally antagonized the effect of *L*-AP4. (d) shows a summary of the above scatter graphs. In all figures traces are from representative slices at i control, ii maximum effect of *L*-AP4, iii 30 min after *L*-AP4 perfusion, iv effect of *L*-AP4 in presence of antagonist and iv 30 min after and of *L*-AP4 + antagonist perfusion.

hippocampal slices from rats 11–18 days old (Fitzjohn *et al.*, 1996).

4-CPG was originally identified as a group I receptor antagonist on the basis that it competitively antagonized ACPD induced stimulation of phosphoinostitide hydrolysis in rat pup cerebral cortex (Birse *et al.*, 1993). In our hands the effect of DHPG was totally inhibited by 4-CPG (400 μM), but unaffected by MCCG (500 μM) or MAP4 (500 μM). DHPG acts upon both mGlu1 and mGlu5 (Lin *et al.*, 1997). 4-CPG appears to be more potent at mGlu1 rather than mGlu5 receptors (Brabet *et al.*, 1995) but the EC_{50} values are so close (44–72 and 150–156 μM respectively) (Kingston *et al.*, 1995) that we could not reliably use this to predict which group I receptor is involved.

The mechanism of the group I mGlu receptor mediated inhibition of the fEPSP could be secondary to depolarization of postsynaptic cells *via* inhibition of K^+ currents (Gereau & Conn, 1995; Davies *et al.*, 1995), but there is also evidence for

presynaptic locus of effect (Fitzjohn *et al*., 1996). A possible mechanism is provided by the observations that in HEK 293 cells group I mGlu receptors decrease the current through N- and P/Q-type voltage gated calcium channels (McCool *et al*., 1998), and that DHPG inhibits 4-aminopyridine induced-glutamate release from hippocampal terminals (Rodriguez-Moreno *et al*., 1998).

Group II mGlu receptors As with DHPG, the effect of a brief perfusion of DCG-IV was highly dependent on the age of rat from which the slices of hippocampus were prepared. A 5-min perfusion of DCG-IV in slices from neonates caused a depression in fEPSP amplitude which was not reversed upon wash out of the drug. The depression in fEPSP slope induced by DCG-IV did not occur immediately on drug contact with the slice but took time to develop, often appearing not to start until drug perfusion had stopped. However if DCG-IV was perfused for 10 min this did not delay the depression in fEPSP. Due to the persistence of the effect it could be termed a form of long-term depression (LTD) (Christie *et al*., 1994). ACPD has previously been reported to induce a form of LTD of neurotransmission at the Schaffer collateral-CA1 synapse in rats 8–12 days old, which could implicate either group I or II mGlu receptors (Overstreet *et al*., 1997). More specifically, in the dentate gyrus DCG-IV induces a reversible depression of fEPSP which is followed by LTD on washout of drug (Huang *et al*., 1999).

Although DCG-IV is a potent, selective group II mGlu receptor agonist (Ishida *et al*., 1994; Cartmell *et al*., 1998), it also binds to ionotropic glutamate receptors (Hayashi *et al*., 1993) and appears to act as an NMDA receptor agonist. However, it is important to note that the concentrations at which it does this are at least 10 fold higher than those used in the present study (Wilsch *et al*., 1994; Breakwell *et al*., 1997).

Evidence that MCCG is an antagonist at group II mGlu receptors includes the following. In slices of spinal cord MCCG (200 μM) inhibited the presynaptic depression of dorsal root evoked monosynaptic excitation of motorneurones evoked by ACPD but not the postsynaptic depolarization produced by ACPD or the presynaptic effect of *L*-AP4 (Jane *et al*., 1994). Similarly, in hippocampal slices MCCG (1 mM) antagonized depressions induced by (1*S*,3*S*)-ACPD, an isomer of ACPD with selectivity for group II mGlu receptors, but not those induced by *L*-AP4 (Schoepp *et al*., 1996). Finally, in cells transfected with mGlu2, MCCG (500 μM) inhibited the action of ACPD but had no effect against the actions of quisqualate or *L*-AP4 in cells expressing mGlu1b or mGlu4 respectively (Knopfel *et al*., 1995). Together these results support the use of MCCG as a group II mGlu receptor antagonist. In our experiments, the perfusion of MCCG (200 μM) had only a minimal effect on DCG-IV induced LTD (data not shown) however, at 500 μM MCCG produced a total inhibition of both acute and long-term depression, and they were unaffected by 500 μM MAP4. These results are consistent with the observations that DCG-IV induced LTD in the dentate gyrus was inhibited by the group II antagonist α-methylserine-O-phosphate monophenyl ester (MSOPPE) (Huang *et al*., 1999), and that MAP4 only antagonized the action of ACPD at mGlu2 receptors at higher concentrations ($IC_{50}=2$ mM) (Knopfel *et al*., 1995). We found that concomitant perfusion of DCG-IV and the proposed group I receptor antagonist, 4-CPG, produced variable results ranging from complete inhibition of depression to a large sustained depression. These inconsistent results could result from the proposed mixed agonist/antagonist action of 4-CPG at group II receptors, such that an antagonistic action of 4-CPG would result in inhibition of the DCG-IV mediated depression whereas, if 4-CPG was acting as an agonist an additive effect may ensue. In Chinese hamster ovary cells expressing mGlu2, 4-CPG reduced forskolin-stimulated cyclic AMP production with an EC_{50} of 970 μM (Cavanni *et al*., 1994; Hayashi *et al*., 1994), whereas, in BHK cells expressing mGlu2 receptors 4-CPG antagonized glutamate evoked inhibition of forskolin stimulated cyclic AMP-formation with an IC_{50} of 577 ± 74 μM but failed to exert any agonistic action towards mGlu2 receptors (Thomsen *et al*., 1994).

The low concentrations of DCG-IV used, together with the selective antagonism by MCCG, suggest that the long-term depression of the fEPSP by DCG-IV is not due to NMDA receptor activation but to stimulation of group II mGlu receptors. The exact nature of this inhibition and what sustains it are yet to be determined but a decrease in neurotransmitter release may be involved (Attwell *et al*., 1998), at least initially.

Group III mGlu receptors In our experiments the group III mGlu receptor agonist *L*-AP4 evoked a reversible depression of the fEPSP amplitude only in slices prepared from neonate rats. This agrees with the reports that the degree of depression of synaptic transmission in CA1 region elicited by *L*-AP4 (50 μM) was inversely correlated with the age of rat from which the slices were prepared (Baskys & Malenka, 1991a).

There are several reports of MAP4 selectively inhibiting responses evoked by *L*-AP4 as opposed to ACPD (Jane *et al*., 1994; Bushell *et al*., 1996; Schoepp *et al*., 1996) and therefore it appears to act as a selective group III antagonist. In our hands, MAP4 at a concentration of 500 μM but not 200 μM totally inhibited the depression in fEPSP amplitude elicited by *L*-AP4, whereas the proposed group II mGlu receptor antagonist, MCCG, was ineffective. The one oddity was the greater depression in synaptic transmission caused by *L*-AP4 in the presence of the proposed group I antagonist, 4-CPG. The available evidence suggests that 4-CPG has no effect on group III receptors (specifically mGlu4) at the concentration used in this study (Thomsen *et al*., 1994). Due to the suggested activation of group II receptors by 4-CPG (described above) an additive effect of group II and group III mediated depressions is possible. However, arguing against this explanation for our results, 4-CPG failed to depress the fEPSP when perfused alone at the concentration used in the antagonist studies (400 μM) or at a higher concentration (800 μM). A synergistic interaction between group I and II has been suggested (Schoepp *et al*., 1996) but no such interactions have, as yet, been reported for group III mGlu receptors. Alternatively, an interaction between group I and group III mGlu receptors could exist such that group I receptors exert a tonic inhibitory effect on group III receptors. An inhibition of group I receptors by 4-CPG would relieve this effect resulting in a greater degree of inhibition by *L*-AP4. This does not seem likely since another proposed group I mGlu receptor antagonist AIDA (Moroni *et al*., 1997) blocked DHPG responses (although not as well as 4-CPG; DHPG 70 ± 2% of control compared with DHPG + AIDA (445 μM) 87 ± 7% of control) but did not augment *L*-AP4 responses (*L*-AP4 74 ± 5% of control, *L*-AP4 + AIDA 68 ± 8% of control). The obvious conclusion is therefore that the effect of 4-CPG in potentiating the depression evoked by *L*-AP4 is not related to its action as a mGlu1 receptor antagonist.

The possibility exists that mGlu5 receptors are responsible since 4-CPG but not AIDA could antagonize these receptors at the concentrations used. Alternatively 4-CPG could be acting indirectly to depress the fEPSP, however if this were the case a depression when perfused alone would be expected or when perfused in combination with other agonists, which is clearly not the case.

As with group II mGlu receptors the proposed mechanism for an inhibition of synaptic transmission by group III receptors is a presynaptically mediated reduction in glutamate release. Evidence in support of this includes a report that the ability of *L*-AP4 to inhibit KCl-induced glutamate release in cerebrocortical terminals declines in week 4 and is non-existent in terminals from adult rats (Vazquez *et al*., 1995). *L*-AP4 also reduced the elevation of cytoplasmic free Ca^{2+} evoked by 4-aminopyridine and KCl. In corticostriatal slices *L*-AP4 and L-SOP decreased EPSP amplitude without affecting the response to focal application of glutamate (Pisani *et al*., 1997).

Correlation with mGlu receptor expression This study has shown that the effects of DHPG, DCG-IV and *L*-AP4 are highly dependent on the stage of animal development, but do these functional effects correlate with receptor expression? Both the location and extent of mGlu receptor expression are very different not only between, but also within, receptor groups.

With regard to the Group I mGlu receptors, the expression of receptor protein for mGlu1a and mGlu5 are both greater at P9 compared to adults, with mGlu1b and c being undetectable at both ages (Casabona *et al*., 1997). In slight contrast, the expression of mGlu1 mRNA increased with age to reach a maximum at postnatal day 30, and although present, the relative level of mGlu1 receptor protein was low with respect to mGlu5 (Condorelli *et al*., 1992). The splice variant mGlu5a presides over mGlu5b in the developing brain with the opposite situation in adults (Romano *et al*., 1996), and a similar expression pattern is found in spinal cord (Valerio *et al*., 1997). *In situ* hybridization using oligonucleotide probes looking at distinct hippocampal regions found mGlu1 levels to increase to adult levels by day 21 with the dentate gyrus and CA3 being more endowed than CA1, whereas mGlu5 was present in all regions to an equally large extent (Catania *et al*., 1994). The majority of studies using electron and light microscopy located both mGlu1 and 5 to the postsynaptic membrane (Lujan *et al*., 1996; Shigemoto *et al*., 1997) with the highest density on dendritic spines (Lujan *et al*., 1996; Romano *et al*., 1996). This, therefore, does not correlate with a presynaptically mediated mechanism for inhibition or potentiation. However some immunoreactivity for both mGlu5 (Romano *et al*., 1996) and mGlu1 (Shigemoto *et al*., 1992) has been detected on presynaptic axon terminals. A correlation exists between mGlu5 receptor expression and functional activity determined as ACPD induced IP_3 formation (Casabona *et al*., 1997). However no such relationship exists for mGlu1 (Condorelli *et al*., 1992; Casabona *et al*., 1997).

Regarding the group II receptors, the relative amount of labelling for mGlu2 and 3 is low in the CA1 region when compared to dentate gyrus and other brain areas with no detection of mGlu2 in adult rats and mGlu3 labelling in CA1 pyramidal cells being only marginally above background levels (Ohishi *et al*., 1993; Catania *et al*., 1994; Fotuhi *et al*., 1994). The lack of effect seen with group II agonists in adults can therefore be correlated with a lack of receptor expression. In general the amount of mGlu2/3 receptor labelling peaked at postnatal day 14 before declining to adult levels (Catania *et al*., 1994).

Of the four members of group III receptors only three are present in the hippocampus since mGlu6 is found almost exclusively in the retina (Nakajima *et al*., 1993). In adult rats low levels of mRNA for mGlu4 is seen in cells in the CA1 region which correlates with a small but detectable level of labelling in this region when using antibodies raised against mGlu4 (Catania *et al*., 1994; Ohishi *et al*., 1995; Phillips *et al*., 1997). In contrast, moderate to high levels of mGlu7 expression were detected throughout the hippocampus with clear amounts in CA1 subfield (Saugstad *et al*., 1994; Ohishi *et al*., 1995; Corti *et al*., 1998), although mGlu8 could also be detected (Saugstad *et al*., 1997; Corti *et al*., 1998). Analysis at the electron microscope level revealed a presynaptic location for mGlu7 in hippocampus (Bradley *et al*., 1996; Shigemoto *et al*., 1997) suggesting that this receptor could mediate the presynaptic actions of *L*-AP4, but this does not exclude a role for mGlu4 or 8. Significantly, the potency of *L*-AP4 at mGlu7 is much less when compared with mGlu4 and 8 with EC_{50} values of 160–500, 0.4–1.2 and 0.4 μM respectively (Conn & Pin, 1997). The lack of effect of *L*-AP4 at 50 μM in adult slices could be explained by a lack of potency and that a much higher concentration would have to be utilized to be effective. However, this does not explain the approximately 40% inhibition caused by *L*-AP4 (50 μM) in the slices from neonates and it is possible that the expression of either mGlu 4 or 8 is substantially greater in the CA1 region of developing hippocampus compared to adult.

Conclusions

Our results have shown that the action of all three groups of mGlu receptors are highly dependent on the age of the animals from which the slices are taken. Agonists for group II and group III receptors have no effect on basal synaptic transmission in slices prepared from young adult rats but in slices from neonates they cause a persistent or short term depression of the fEPSP respectively. An agonist for group I receptors caused a transient depression of the fEPSP in neonatal tissue, but a slow onset potentiation in adult tissue. These observations go some way to explaining inconsistencies between previously reported results.

The existing literature describing the developmental regulation of expression of specific receptors is too incomplete to draw any firm conclusions beyond the fact that the lack of effect of the group II agonist correlates with a lack of expression of mGlu 2 or 3 in adult tissue.

Investigation of three proposed antagonists showed that MCCG and MAP4 are effective and selective group II and group III receptor antagonists respectively. 4-CPG appeared to be an effective group I receptor antagonist but it's usefulness as a pharmacological tool may be complicated by an effect on both group II and group III receptor mediated responses.

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