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AGONISTS AT METABOTROPIC GLUTAMATE RECEPTORS PRESYNAPTICALLY INHIBIT EPSCs IN NEONATAL RAT HIPPOCAMPUS

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SUMMARY

1. The effects of metabotropic glutamate receptor agonists on excitatory synaptic transmission in the CA1 region of rat hippocampal slices (11–30 days) were studied using extracellular and whole-cell patch-clamp recording techniques.

2. Trans-1-amino-1,3-cyclopentanedicarboxylic acid (trans-ACPD; 25–100 μM) reversibly depressed excitatory postsynaptic currents (EPSCs) without affecting presynaptic fibre excitability or EPSC reversal potential.

3. Ibotenate (25 μM) or L-glutamate (250 μM), in the presence of the *N*-methyl-D-aspartate (NMDA) receptor antagonist, D-2-amino-5-phosphonovaleric acid (APV, 50–75 μM), depressed the EPSC amplitude while inducing no detectable inward current. L-2-Amino-4-phosphonobutyrate (L-AP4, 25–100 μM), the phosphonic derivative of glutamate, also depressed EPSC amplitude and caused no detectable inward current.

4. The NMDA receptor-mediated component of the EPSC recorded in the presence of the non-NMDA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20–30 μM) was depressed by trans-ACPD, L-AP4, or quisqualate (1–2 μM).

5. The response to ionophoretic application of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) was unaffected by trans-ACPD or L-AP4 although the simultaneously recorded EPSC was strongly depressed. In addition, paired-pulse facilitation (50–75 ms interstimulus interval) was reversibly enhanced by trans-ACPD or L-AP4. These results indicate that the depression of synaptic transmission likely was mediated by a presynaptic 'autoreceptor'.

6. The effects of trans-ACPD or L-AP4 on synaptic transmission decreased significantly over ages 12–30 days and were minimal in adult (> 80 days) slices.

7. The depression of synaptic transmission caused by trans-ACPD or L-AP4 was not altered following the induction of long-term potentiation (LTP).

8. The results indicate that metabotropic glutamate receptor agonists suppress excitatory synaptic transmission in CA1 pyramidal cells by an action at a presynaptic

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site. This effect is developmentally regulated and is maximally expressed during the first postnatal month.

INTRODUCTION

Experiments using pharmacological, biochemical, electrophysiological and recently molecular techniques have permitted a reasonably detailed classification of excitatory amino acid receptors (Collingridge & Lester, 1989; Monaghan, Bridges & Cotman, 1989). Because of their experimental accessibility, postsynaptic receptors have been most extensively characterized and commonly are divided into AMPA (α-amino-3-hydroxy-5-methyl-isoxazole propionic acid) (or quisqualate), kainate, and NMDA (*N*-methyl-D-aspartate) receptors, named according to their characteristic agonists. These receptors are ligand-gated ion channels (ionotropic receptors) and a great deal is known about their biophysical properties and, for some non-NMDA receptors, their molecular structures (Barnard & Henley, 1990). In contrast, much less is known about two more recently characterized glutamate receptors; the so-called L-AP4 (L-2-amino-4-phosphonobutyrate) (L-APB) and metabotropic glutamate (quisqualate) receptors.

Metabotropic glutamate receptors were initially revealed by two observations: (1) excitatory amino acids stimulated phosphoinositide (PI) turnover in striatal neurones in culture (Sladeczek, Pin, Recasens, Bockaert & Weiss, 1985) and (2) following injection of rat brain mRNA into *Xenopus* oocytes, glutamate caused oscillatory inward currents due to activation of PI turnover (Sugiyama, Ito & Hirono, 1987). Several glutamate receptor agonists act on the metabotropic receptor (Schoepp, Bockaert & Sladeczek, 1990) and recently, trans-1-amino-1,3-cyclopentanedicarboxylic acid (trans-ACPD), has been reported to be a specific agonist at this receptor (Palmer, Monaghan & Cotman, 1989; Manzoni, Fagni, Pin, Rassendren, Poulatt, Sladeczek & Bockaert, 1990). In hippocampal slices, stimulation of PI turnover by metabotropic glutamate receptors is greatest in the early phases of postnatal development and is significantly reduced in adult animals (Nicoletti, Iadarola, Wroblewski & Costa, 1986; Palmer, Nangel-Taylor, Krause, Roxas & Cotman, 1990). The L-AP4 receptor was identified by the ability of L-AP4 to suppress synaptic transmission at certain central nervous system (CNS) synapses (see Collingridge & Lester, 1989; Monaghan *et al.* 1989). Electrophysiological studies have demonstrated that the L-AP4 receptor is likely a presynaptic 'autoreceptor' which functions to inhibit transmitter release (Harris & Cotman, 1983; Cotman, Flatman, Ganong & Perkins, 1986; Forsythe & Clements, 1990) although the mechanism by which this occurs is unclear.

Recently, the effects of activation of the metabotropic glutamate receptor on postsynaptic ionic conductances and excitability have been examined (Stratton, Worley & Baraban, 1989; Baskys, Bernstein, Barolet & Carlen, 1990; Charpak, Gahwiler, Do & Knopfel, 1990). The study presented here was undertaken to examine whether metabotropic glutamate receptor stimulation directly modulates synaptic transmission, independent of effects on postsynaptic ionic conductances. To address these questions, extracellular field potential and whole-cell patch-clamp recording techniques were used to record excitatory postsynaptic currents (EPSCs) generated in CA1 pyramidal cells by stimulation of the Schaffer collateral/

commissural afferents in hippocampal slices prepared from young (11–30 days) rats. Preliminary accounts of part of this work have been published elsewhere (Baskys & Malenka, 1991; Malenka & Baskys, 1991).

METHODS

Rat hippocampal slices (400 μm) were prepared using standard techniques (Nicoll & Alger, 1981; Malenka, Kauer, Zucker & Nicoll, 1988) and placed in a holding chamber for at least 1 h. Animals were deeply anaesthetized with halothane and decapitated with a guillotine. The age of rats from which slices were prepared turned out to be a critical variable in this study (see Results). Unless stated otherwise, all recordings were made from slices prepared from rats aged 11–30 days. For recording, one slice was transferred to the recording chamber where it was submerged beneath a continuously superfusing solution that had been saturated with 95% O_2 and 5% CO_2. The composition of the solution was (in mM): NaCl, 119; KCl, 2·5; $MgSO_4$, 1·3; NaH_2PO_4, 1·0; $NaHCO_3$, 26·2; $CaCl_2$, 2·5; glucose, 11. When picrotoxin (30 μM) was added to the solution, the CA3 region was surgically removed. The temperature of the solution was maintained between 28 and 30 °C.

Extracellular field excitatory postsynaptic potentials (EPSPs) were recorded in stratum radiatum with electrodes (2–6 MΩ) filled with 3 M-NaCl. 'Blind' (Blanton, Lo Turco & Kriegstein, 1989; Coleman & Miller, 1989) whole-cell patch-clamp recordings were made from CA1 pyramidal cells with electrodes (1–4 MΩ) filled with one of two solutions. Solution 1 contained (in mM): CsF, 130; NaCl, 10; EGTA, 10; HEPES, 10, pH = 7·2. Solution 2 contained (in mM): potassium gluconate, 117·5; potassium methyl sulphate, 20; NaCl, 8; HEPES, 10; EGTA, 0·1; Mg-ATP, 2; GTP, 0·2; pH = 7·2. Voltage clamp recordings were made using the continuous mode of an Axoclamp-2A amplifier (Axon Instruments). During synaptic stimulation cells were held between −65 and −85 mV. To elicit synaptic currents, Schaffer collateral/commissural afferents in stratum radiatum were stimulated at 0·1 Hz with bipolar stainless-steel electrodes. In most experiments, the stimulating electrodes were placed within 50–75 μm of the soma in order to activate synapses close to the soma and thus improve the adequacy of the voltage clamp (Hestrin, Nicoll, Perkel & Sah, 1990).

Data were collected and analysed on-line (3–10 kHz sampling rate) using Axobasic (Axon Instruments). All illustrated data traces are averages of two to six successive sweeps. Drugs were added to the bathing solution immediately prior to application to the slice. A peristaltic pump (2 ml/min) was used to circulate the solution through the recording chamber (volume approximately 0·5 ml). This kept the flow rate constant and avoided any flow artifacts. Ionophoretic microelectrodes were filled with α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA, 20 mM in 150 mM-NaCl) and positioned in stratum radiatum so as to evoke a brisk inward current with moderate (50–150 nA) ionophoretic currents. Drugs used included: trans-1-amino-1,3-cyclopentanedicarboxylic acid (trans-ACPD; Tocris Neuramin), L-2-amino-4-phosphonobutyrate (L-AP4; Cambridge Research Biochemicals), D-2-amino-5-phosphonovaleric acid (D-APV; Cambridge Research Biochemicals), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Cambridge Research Biochemicals), quisqualate (Sigma), ibotenate (Sigma), L-glutamate (Sigma), picrotoxin (Sigma), *N*-methyl-D-aspartate (NMDA; Sigma).

RESULTS

The results reported in this paper are based on field EPSPs recorded from sixty-four slices and whole-cell EPSCs obtained from sixty-one pyramidal cells. Input resistance of these cells ranged from 180 to 350 MΩ; series resistance ranged from 7–48 MΩ. Field EPSPs were 0·4–1·0 mV in amplitude; whole-cell EPSCs were 100–700 pA.

Metabotropic glutamate receptor agonists and synaptic transmission

Trans-ACPD (25–100 μM) reversibly depressed the field EPSP in a dose-dependent manner ($n = 28$; Fig. 1*A*). At a concentration of 50 μM, trans-ACPD depressed the

field by 48 ± 19 % ($n = 18$). This effect exhibited no evidence of desensitization even when trans-ACPD was applied for periods up to 15 min. A high concentration of trans-ACPD (500 μM) depressed the field EPSP by greater than 90 % ($n = 3$). To determine whether decreases in the number of presynaptic fibres activated might

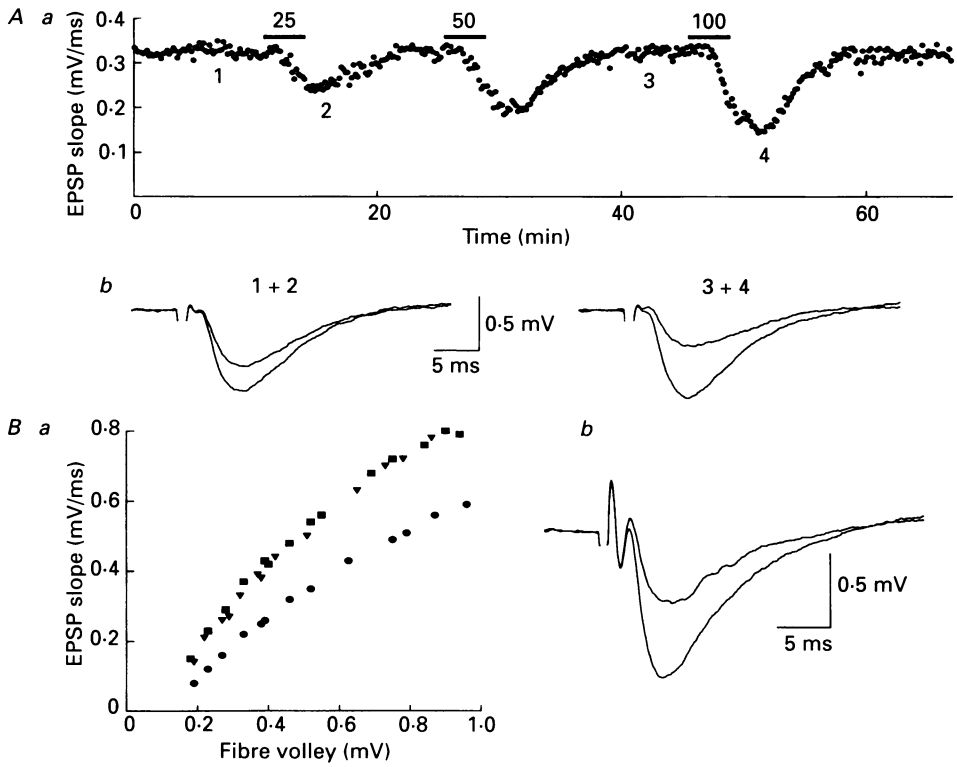

Fig. 1. Effect of trans-ACPD on field EPSP and presynaptic fibre volley. *Aa*, plot of the field EPSP initial slope during extracellular bath application of increasing concentrations of trans-ACPD. In this and all subsequent figures, the dark bar above the graph indicates the time at which drug application occurred. The numbers above the bars indicate the concentration of trans-ACPD (μM) applied. *Ab*, sample field EPSPs taken at the time indicated by the numbers on the graph. *Ba*, graph of field EPSP slope as a function of fibre volley amplitude before (■), during (●) and following (▼) application of 50 μM-trans-ACPD. *Bb*, sample traces taken from this experiment showing that trans-ACPD had no detectable effect on fibre volley amplitude or latency.

account for the synaptic depression, we compared the field EPSP with the presynaptic fibre volley by constructing input–output curves. Trans-ACPD shifted this curve to the right and had no discernible effect on the shape or size of the fibre volley (Fig. 1*B*). These data indicate that changes in presynaptic axonal excitability cannot account for the actions of trans-ACPD on synaptic transmission.

Trans-ACPD reversibly depressed the EPSC without any change in the holding current when cells were voltage clamped with CsF-containing electrodes and held at −70 to −80 mV ($n = 6$; see Baskys & Malenka, 1991). Trans-ACPD (25–50 μM) also depressed synaptic transmission when applied in the presence of the specific NMDA receptor antagonist D-APV (50 μM; $n = 3$) indicating that its actions were not

mediated by NMDA receptors. When the recording electrodes containing potassium gluconate-based solution 2, trans-ACPD again depressed the EPSC ($n = 12$) although some inward current (10–60 pA) was generated.

The depression of synaptic transmission by trans-ACPD exhibited no voltage dependence and did not significantly affect the EPSC reversal potential ($n = 3$;

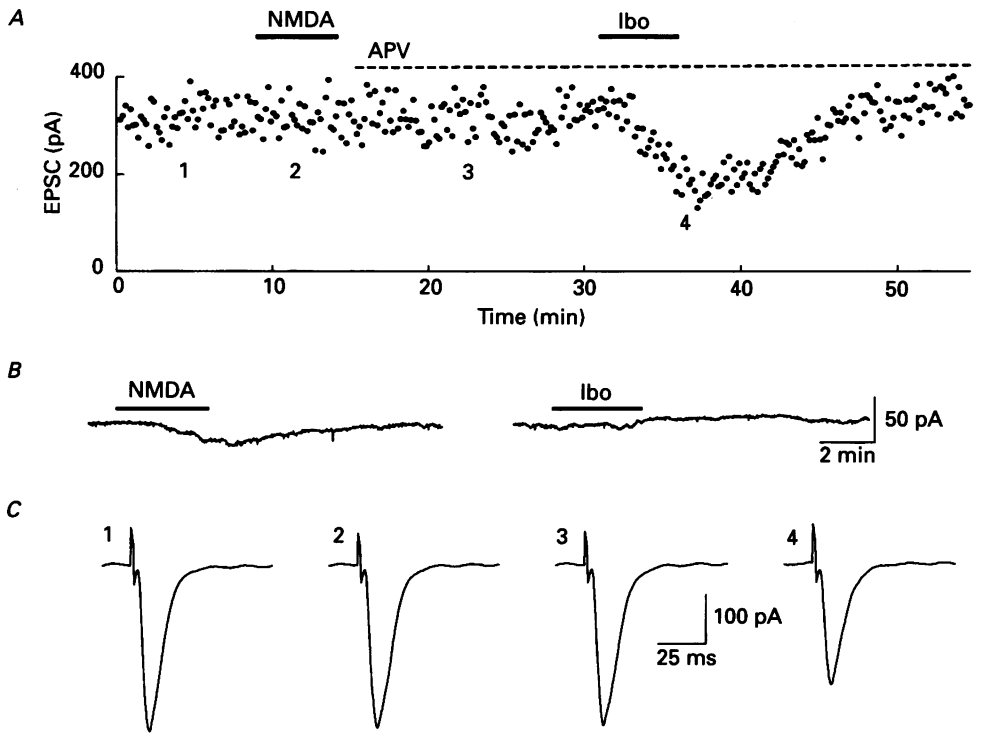

Fig. 2. Ibotenate depresses synaptic transmission independent of its action on NMDA receptors. *A*, plot of EPSC amplitude from an experiment which demonstrates that NMDA (1 μM) had no effect on EPSC amplitude whereas ibotenate (25 μM), applied in the presence of D-APV (75 μM) reversibly depressed EPSC amplitude. *B*, traces of changes in holding current demonstrating that in this cell, NMDA caused a modest inward current while ibotenate had no detectable effect. *C*, sample EPSCs taken at the times indicated by the numbers on the graph.

control reversal potential $= -7{\cdot}6 \pm 6{\cdot}9$ mV; reversal potential in trans-ACPD $= -5{\cdot}7 \pm 3{\cdot}9$ mV). Synaptic conductance was clearly decreased by trans-ACPD as evidenced by a decrease in the slope of the current–voltage relation.

To determine whether trans-ACPD was acting on a receptor with pharmacological properties similar to those of the metabotropic glutamate receptor, the effects on EPSCs of two reasonably potent metabotropic glutamate receptor agonists, ibotenate and quisqualate (Schoepp *et al.* 1990), were tested. However both of these compounds also act on ionotropic receptors; ibotenate activates NDMA receptors and quisqualate activates AMPA receptors (Collingridge & Lester, 1989). Figure 2 shows a comparison of the effects of NMDA and ibotenate on the EPSC. NMDA (0·5–1·0 μM) had no significant effect on synaptic transmission ($n = 5$) even though it had clear postsynaptic actions (Fig. 2*B*). In contrast, ibotenate (25 μM) in the

presence of D-APV (75 μM), depressed the EPSC (50·9 ± 17 %; $n = 5$) while causing no significant change in the holding current. The actions of quisqualate on synaptic transmission could not be determined (but see below) since under normal recording conditions, the early portion of the EPSC is predominantly mediated by AMPA receptors (Hestrin *et al.* 1990; Randall, Schofield & Collingridge, 1990).

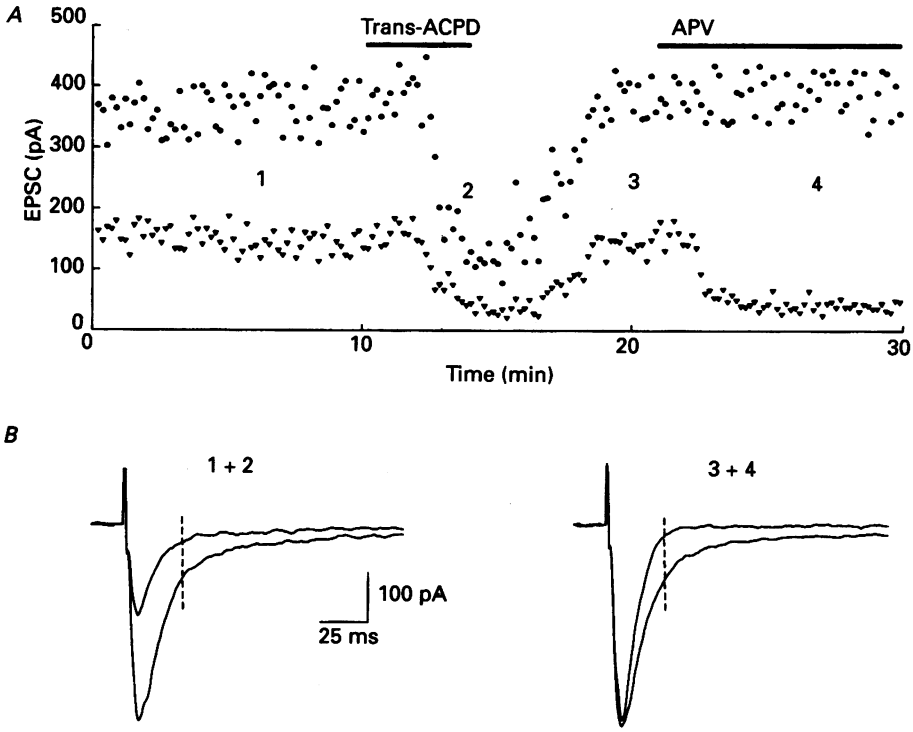

Fig. 3. Trans-ACPD depresses both the early and late components of the EPSC. *A*, plot of EPSC amplitude measured at the peak (●) and 25 ms after the peak (▼) during trans-ACPD (100 μM) application and then D-APV (25 μM) application. Perfusate contained 30 μM-picrotoxin and the cell was held at −50 mV. Trans-ACPD reversibly depressed both the presumptive AMPA-mediated and NMDA-mediated components of the EPSC whereas D-APV only depressed the late, NMDA-meditated component. *B*, traces taken at the times indicated by the numbers on the graph. Dashed line marks the time at which the late component was measured.

The effects of glutamate, the likely endogenous ligand for the metabotropic glutamate receptor, were also examined. In initial attempts, concentrations of glutamate (200–400 μM) which depressed synaptic transmission caused an inward current making the measurement of the EPSC unreliable. Because the NMDA receptor has a much higher affinity for glutamate than ionotropic non-NMDA receptors (Collingridge & Lester, 1989), we repeated the experiments in the presence of D-APV (50 μM) to determine whether this could prevent the glutamate-induced inward current. In five cells glutamate (250 μM) caused a clear, reversible depression of the EPSC while causing no detectable inward current.

The actions of trans-ACPD, ibotenate and glutamate on synaptic transmission are similar to the previously reported effects of L-AP4 (Koerner & Cotman, 1981; Davies

& Watkins, 1982; Harris & Cotman, 1983; Cotman *et al.* 1986; Nawy, Sie & Copenhagen, 1989; Forsythe & Clements, 1990), a structurally similar, phosphonic derivative of glutamate (Collingridge & Lester, 1989; Monaghan *et al.* 1989). L-AP4 has also been reported to stimulate PI turnover (Schoepp *et al.* 1990) indicating that

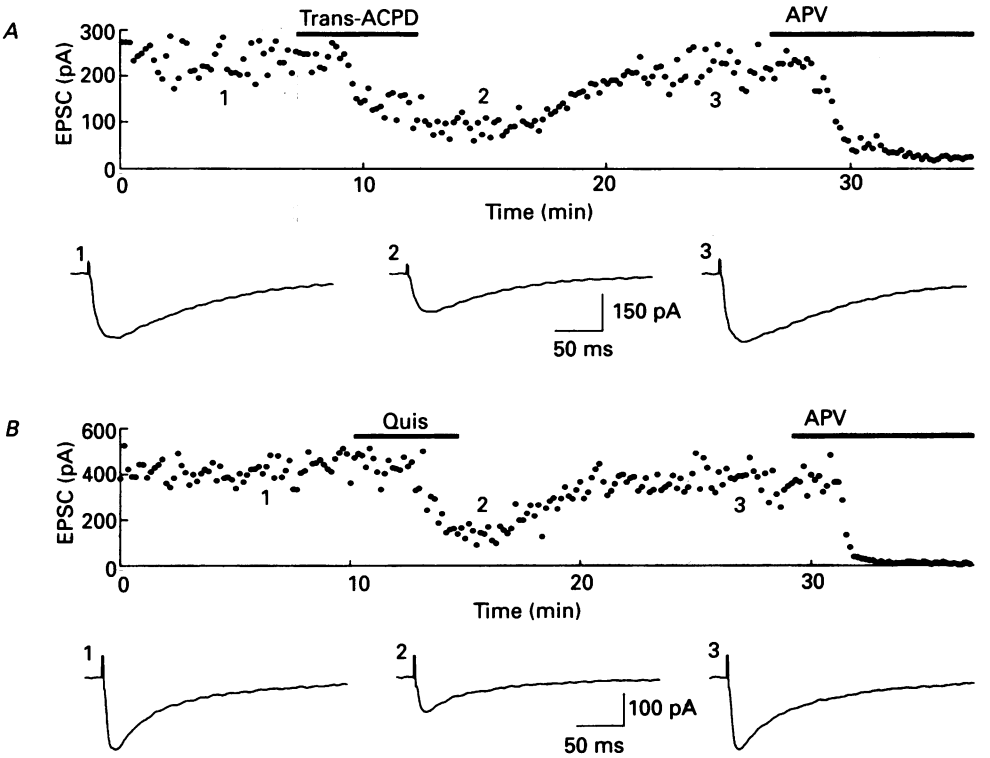

Fig. 4. Trans-ACPD or quisqualate depress the NMDA receptor-mediated component of the EPSC recorded during blockade of AMPA receptors. Perfusate contained 20 μM-CNQX, 30 μM-picrotoxin, and no added magnesium. Graphs of EPSC amplitude from experiments in which trans-ACPD (75 μM, *A*) or quisqualate (2 μM, *B*) was applied. In both experiments, D-APV (25 μM) completely blocked the EPSC, indicating that it was mediated by NMDA receptors. Sample traces taken at the times indicated by the numbers are shown below each graph.

it can interact with metabotropic glutamate receptors. L-AP4 (25–100 μM) reversibly depressed both field EPSPs ($n = 17$) and whole-cell EPSCs ($n = 9$) while having no consistent effects on leak conductance or holding current. A concentration of 50 μM-L-AP4 caused a $32{\cdot}6 \pm 15\%$ decrease in the field EPSPs ($n = 12$) and a $30{\cdot}5 \pm 13{\cdot}5\%$ in the EPSCs ($n = 5$). High concentrations (1–2 mM) of L-AP4 depressed the field EPSP by 70–95% ($n = 4$).

Effects on the NMDA component of the EPSC

Both functionally and mechanistically, an important question is whether metabotropic glutamate receptor agonists suppress the NMDA as well as the AMPA receptor-mediated component of the EPSC. Holding the cell between −55 and

−45 mV in the presence of picrotoxin it is possible to record an EPSC with contributions from both components (Hestrin *et al.* 1990). Figure 3 shows that trans-ACPD simultaneously depressed both components of the EPSC to approximately the same degree while D-APV only suppressed the late NMDA receptor-mediated component ($n = 4$).

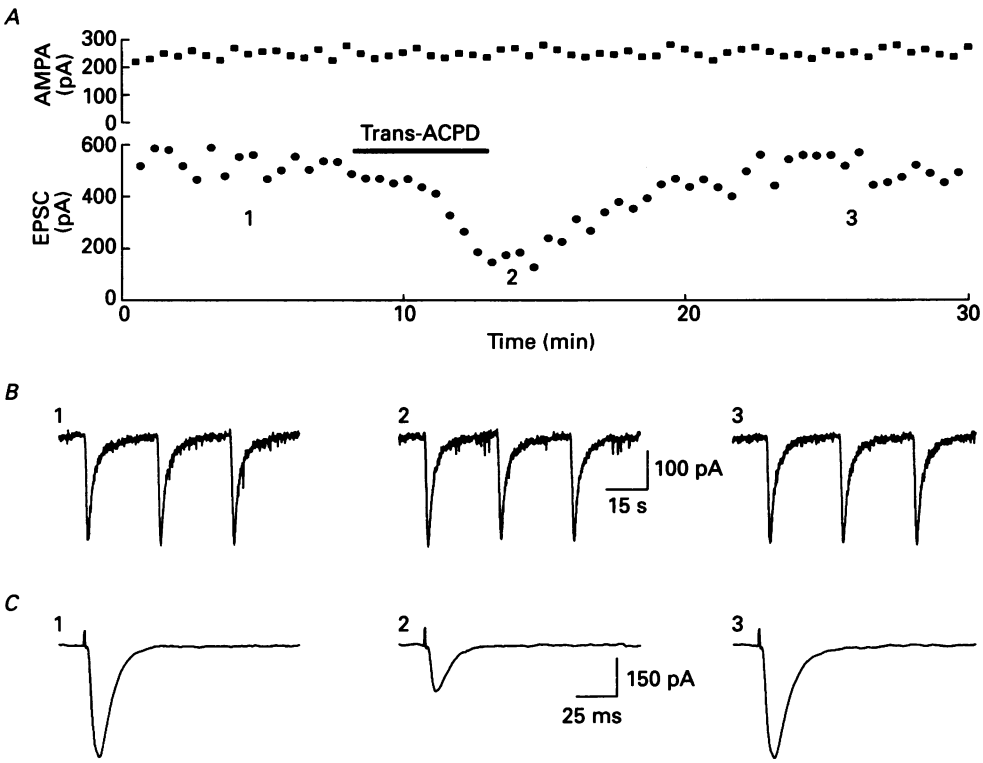

Fig. 5. The responses to ionophoretically applied AMPA are not effected by trans-ACPD. *A*, graph of the amplitude of inward currents generated by ionophoretic application of AMPA (150 nA, 1 s) and EPSC amplitude simultaneously recorded during an experiment in which trans-ACPD (100 μM) was applied. The AMPA responses were unaffected while the EPSC was strongly depressed. Sample AMPA responses (*B*) and EPSCs (*C*) taken at the times indicated by the numbers on the graph.

To examine more directly the effects of trans-ACPD and L-AP4 on the NMDA receptor-mediated component of the EPSC, EPSCs were recorded in the presence of picrotoxin, the AMPA receptor antagonist, CNQX (20 μM) and in nominally Mg^{2+}-free solution. Under these conditions, a large, long-lasting EPSC mediated by NMDA receptors can be recorded (Hestrin *et al.* 1990). Both trans-ACPD($n = 8$) and L-AP4 ($n = 5$) reversibly depressed this EPSC (Fig. 4*A*). The presence of CNQX also permitted an examination of the effects of quisqualate on synaptic transmission. Figure 4*B* shows that without an AMPA receptor-stimulated inward current, quisqualate (2 μM) reversibly depressed the NMDA receptor-mediated EPSC ($n = 5$).

Determination of pre- or postsynaptic site of action

To directly test whether the sensitivity of postsynaptic receptors is modified by these compounds, responses to exogenously applied AMPA were recorded during the application of trans-ACPD or L-AP4. Figure 5 shows that at a time when the EPSC

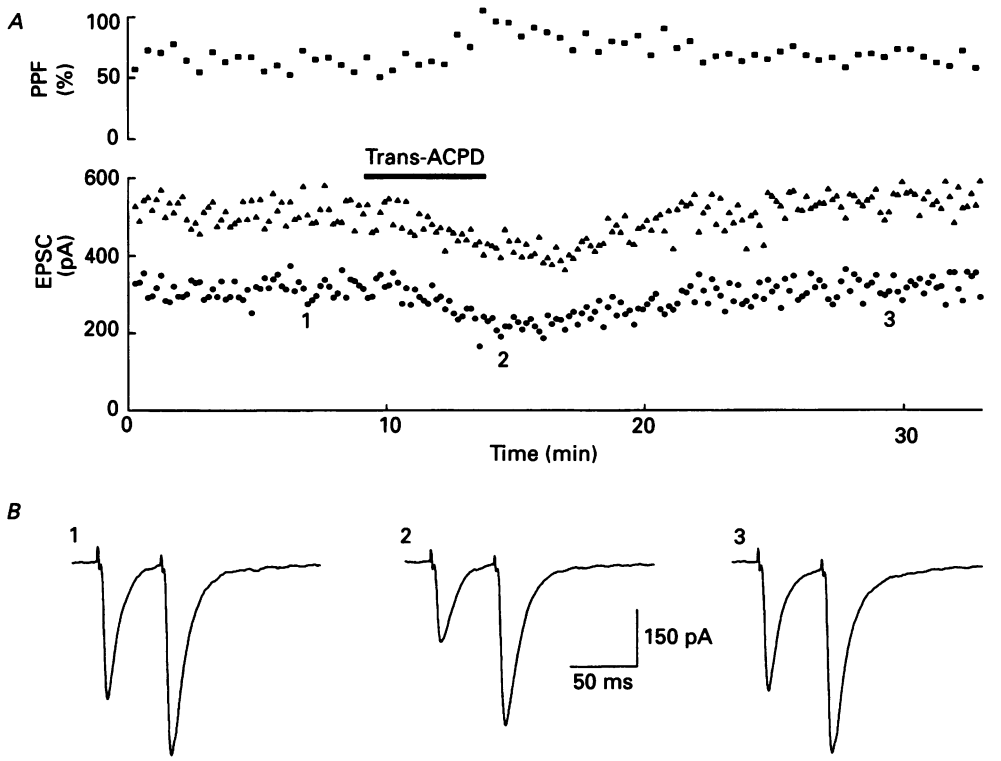

Fig. 6. Paired-pulse facilitation is enhanced by trans-ACPD. *A*, graphs of the EPSC amplitude in response to either the first (●) or second (▲) of two stimuli (interstimulus interval = 50 ms) from an experiment in which trans-ACPD (50 μM) was applied. The top graph plots the percentage facilitation of the second response (100% indicates second response is twice as large as first response). Each point is the average of three successive measurements. *B*, sample data traces taken at the times indicated by the numbers on the graph.

was strongly depressed by trans-ACPD (100 μM), responses to ionophoretically applied AMPA were completely unaffected. In all cells tested (three with trans-ACPD; two with L-AP4), the AMPA responses were not affected by these agonists while the EPSC was depressed by 57·5 ± 15%.

An alternative method by which to determine whether the receptor is localized pre- or postsynaptically involved examining the effects of trans-ACPD or L-AP4 on paired-pulse facilitation. Using field recordings and monitoring paired-pulse facilitation with every stimulus (0·1 Hz) trans-ACPD and L-AP4 enhanced paired-pulse facilitation (measured at 50–75 ms interstimulus intervals) in sixteen of nineteen and nine of eleven slices, respectively. However, in these conditions, the

large magnitude of the EPSP in response to the second pulse may result in a decrease in driving force and non-linear summation, thus inhibiting the full expression of the facilitation (Martin, 1955). This problem can be avoided by using whole-cell recording and voltage clamping. Figure 6 shows that under these recording

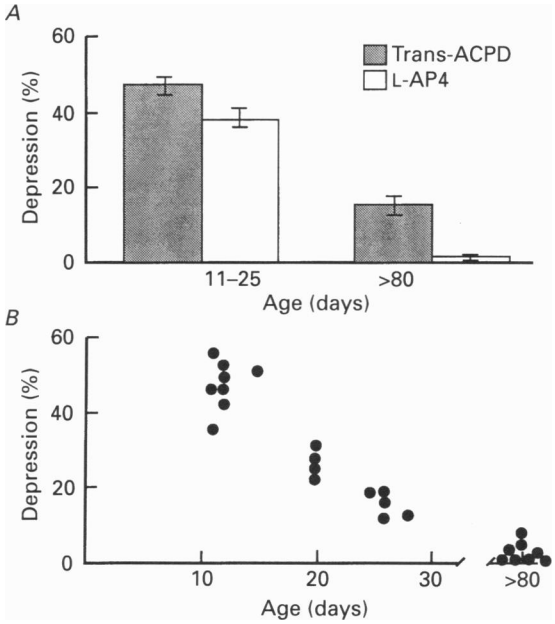

Fig. 7. The depression of synaptic transmission by trans-ACPD and L-AP4 is developmentally regulated. *A*, summary of experiments in which 50 μM-trans-ACPD or L-AP4 was applied for exactly 5 min in slices prepared from animals of different ages. Both trans-ACPD and L-AP4 had minimal ($< 15\%$) or no effects in adult slices. *B*, graph of the depression of synaptic transmission caused by 50 μM-L-AP4 as a function of the age of the animal from which slices were prepared. The effectiveness of L-AP4 decreased dramatically from 11–30 days ($r = -0{\cdot}86$).

conditions, trans-ACPD (50–100 μM) still enhanced paired-pulse facilitation (in five of six cells). Similar results were observed when L-AP4 (100 μM) was applied (seven of seven cells). This enhancement of paired-pulse facilitation is consistent with a presynaptic locus for the action of L-AP4 and trans-ACPD.

Developmental course of the synaptic effects of L-AP4 and trans-ACPD

In hippocampal slices, the stimulation of phosphoinositide turnover due to activation of metabotropic glutamate receptors decreases substantially as the animals age from 6 to 30 days (Nicolleti *et al.* 1986; Palmer *et al.* 1990). To determine whether the inhibition of synaptic transmission reported here also was affected during development we compared the effects of a fixed concentration (50 μM) of L-AP4 or trans-ACPD on synaptic transmission in slices prepared from adult (age 80–150 days) and young (11–25 days) animals. L-AP4 had no effect and trans-ACPD had minimal effect on synaptic transmission in adult slices (Fig. 7*A*). The residual effect of trans-ACPD may be due to its direct depolarizing actions on CA1 pyramidal

cells. Figure 7*B* shows that the effectiveness of L-AP4 (which as mentioned above had no direct postsynaptic effects) was strongly (correlation coefficient (r) = −0·86) inversely correlated with the age of the animal.

Effect of LTP on synaptic actions of trans-ACPD and L-AP4

An increase in transmitter release may occur during long-term potentiation (LTP) (Dolphin, Errington & Bliss, 1982; Bekkers & Stevens, 1990; Malinow & Tsien,

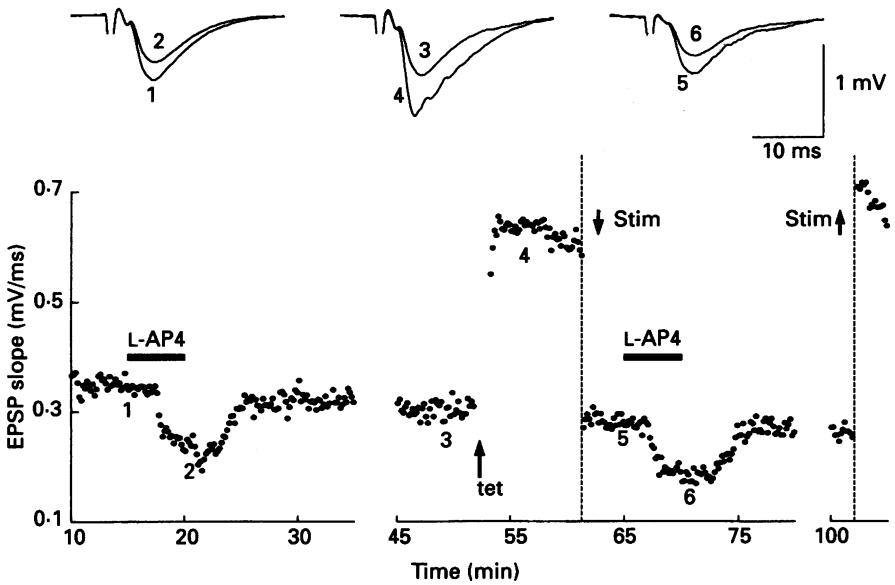

Fig. 8. LTP does not alter the depression of synaptic transmission caused by L-AP4. Following L-AP4 (50 μM) application, LTP was induced with a tetanus (tet: 100 Hz, 1 s given twice separated by 30 s). Stimulation strength was decreased (↓Stim) to approximately match the original EPSP slope and L-AP4 was again applied. At the end of the experiment the stimulus strength was increased (Stim↑) to its original value to show that LTP was still maintained. The data traces were taken at the times indicated by the numbers on the graph.

1990). To test whether a decrease in the number or functional efficacy of presynaptic 'autoreceptors' occurs during LTP, an agonist (L-AP4 or trans-ACPD) was applied before and after the generation of LTP. Figure 8 shows that the L-AP4-induced depression of synaptic transmission was not significantly altered during LTP. Similar results were obtained in four experiments (L-AP4, 50 μM, $n = 2$; trans-ACPD, 50 μM, $n = 2$).

DISCUSSION

The presented experiments demonstrate that metabotropic glutamate receptor agonists depress synaptic transmission in area CA1 of hippocampal slices. The depressant action of these agents was not due to indirect postsynaptic electrophysiological effects (e.g. depolarization) or to direct interaction with post-

synaptic ionotropic non-NMDA or NMDA receptors mediating the recorded EPSCs. The observed effects were robust in slices prepared from young animals but were minimal or absent in slices prepared from adult animals.

Although studies indicate that trans-ACPD is highly selective for the metabotropic receptor (Palmer *et al.* 1989; Manzoni *et al.* 1990), it may have some weak direct action on ionotropic receptors (Magnuson, Curry, Peet & McLennan, 1988; Schoepp *et al.* 1990). Whole-cell recording demonstrated that trans-ACPD could depress synaptic transmission in the absence of any change in holding current. The metabotropic glutamate receptor agonists, ibotenate, quisqualate, and glutamate, (Schoepp *et al.* 1990) all also depressed the EPSC. Trans-ACPD, ibotenate, and glutamate retained this action in the presence of D-APV and did not generate any detectable inward current. Furthermore, NMDA generated inward current but had no significant effect on synaptic transmission. These findings are similar to those found in hippocampal culture (Forsythe & Clements, 1990) and indicate that it is unlikely that the depressant effect on synaptic transmission of metabotropic glutamate receptor agonists is due to activation of ionotropic non-NMDA or NMDA receptors. (These experiments, however, do not rule out the possibility that there are presynaptic NMDA receptors which modulate transmitter release (Chernevskaya, Obukhov & Krishtal, 1991) and which have a lower affinity for NMDA than do postsynaptic NMDA receptors.)

L-AP4, a compound which can function as a weak agonist at metabotropic glutamate receptors (Schoepp & Johnson, 1988; Schoepp *et al.* 1990), also depressed synaptic transmission in area CA1. This finding makes the subtyping of the receptor mediating the effects reported here difficult. Examining if the effects of trans-ACPD and L-AP4 on synaptic transmission were additive could provide information about whether the agonists act on the same or distinct receptors. This experiment could not be performed accurately because high concentrations of either compound depressed EPSPs by over 90%. At other synaptic pathways, glutamate receptors that depress synaptic transmission uniformly have been identified as L-AP4 receptors (Collingridge & Lester, 1989; Monaghan *et al.* 1989). However until the development of specific antagonists for the L-AP4 or the metabotropic glutamate receptor, we cannot determine which receptor subtype is responsible for modulating synaptic transmission in area CA1 of rat hippocampus.

Several lines of evidence strongly support the hypothesis that the receptor mediating the depression of synaptic transmission is localized presynaptically. First, both the AMPA and NMDA receptor-mediated components of the EPSC were depressed by metabotropic glutamate receptor agonists. Although this finding does not rule out a postsynaptic site of action, it is most easily explained by a reduction in transmitter release. Second, the responses to exogenous agonist (AMPA) were not affected at a time when the EPSC was strongly decreased. Third, paired-pulse facilitation, a presynaptic phenomenon, was enhanced during the reduction of the EPSC. In other synaptic pathways where the issue has been examined, the evidence also strongly favours a presynaptic site of action for L-AP4 (Harris & Cotman, 1983; Cotman *et al.* 1986; Forsythe & Clements, 1990).

The mechanism by which this presumptive presynaptic "autoreceptor' reduces transmitter release is unknown. Quisqualate depresses calcium currents in cultured

hippocampal neurones probably through a metabotropic receptor (Lester & Jahr, 1990). Such an effect in the presynaptic terminal might cause a reduction in transmitter release if the quisqualate-modulated calcium channels contribute to the calcium influx necessary for transmitter release. Alternatively, if as occurs in dentate granule cells (Baskys *et al.* 1990) and in CA1 cells (Stratton *et al.* 1989; Charpak *et al.* 1990), activation of this receptor depolarizes the presynaptic terminal, a reduction in evoked transmitter release may follow.

The increase in PI turnover mediated by the metabotropic glutamate receptor decreases markedly during development (Palmer *et al.* 1990; Schoepp *et al.* 1990). For example, in 30-day-old hippocampal slices the stimulation of PI turnover by quisqualate (10 μM) is about 20–25% that observed in 9-day-old slices (Palmer *et al.* 1990). We have found that the depression of synaptic transmission caused by trans-ACPD or L-AP4 is markedly less in adult slices. This raises the possibility that the presynaptic receptors mediating the depression in synaptic transmission are also the receptors which elicit increased PI turnover.

Previous investigators have reported that moderate concentrations of L-AP4 (100–200 μM) have no effects on synaptic transmission in CA1 pyramidal cells (Robinson, Whittemore, Marks & Koerner, 1986; Harris, Stevens & Cotman, 1987) (although higher concentrations have strong depressant effects; Dunwiddie, Madison & Lynch, 1978; Koerner & Cotman, 1982). The developmental changes in the efficacy of L-AP4 likely explain this discrepancy since previous studies were done primarily in slices prepared from adult animals.

A presynaptic glutamate receptor may modulate synaptic transmission in several different ways depending on its K_D, the ambient concentration of glutamate, and the rate at which synaptically released glutamate is removed from the microenvironment (Forsythe & Clements, 1990). The control of excitatory synaptic transmission by presynaptic 'autoreceptors' may be particularly important during development when synaptic connections are unstable and uptake mechanisms may not be completely developed. One intriguing possibility is that changes in the function of presynaptic glutamate receptors contribute to the increase in synaptic efficacy during long-term potentiation (LTP). However, we have found in the CA1 region that the decrease in synaptic transmission caused by L-AP4 or trans-ACPD is unchanged following the generation of LTP.

Presynaptic glutamate receptors activated by L-AP4 have been shown to inhibit excitatory synaptic transmission at several other synapses (Koerner & Cotman, 1981; Davies & Watkins, 1982; Harris & Cotman, 1983; Cotman *et al.* 1986; Nawy *et al.* 1989; Forsythe & Clements, 1990). It will be interesting to determine whether, as these synapses, metabotropic glutamate receptor agonists have similar inhibitory actions on synaptic transmission. A rigorous examination of the functional role of these presynaptic autoreceptors will require the development of highly specific receptor antagonists. With such compounds, it should be possible to determine the role these receptors play during low- and high-frequency synaptic transmission

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