Presynaptic Depression of Synaptic Transmission Mediated by Activation of Metabotropic Glutamate Receptors in Rat Neocortex

James P. Burke and John J. Hablitz

Neurobiology Research Center and Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama 35294

Conventional intracellular recordings were obtained from layer II–III neurons in adult rat neocortical brain slices. Excitatory and inhibitory (I) postsynaptic potentials (PSPs) were evoked prior to and during bath application of agonists and antagonists of metabotropic glutamate receptors (mGluRs). In the presence of the selective mGluR agonist 1S,3R-1-aminoctane-1,3-dicarboxylic acid (1S,3R-ACPD; 5–200 μM), both excitatory and inhibitory components of the evoked PSPs were reversibly reduced. PSPs were significantly, but less effectively, decreased by L-2-amino-4-phosphonobutyric acid. Exposure to putative mGluR antagonists, α-methyl-4-carboxyphenylglycine or L-2-amino-3-phosphonopropionic acid, did not inhibit the 1S,3R-ACPD–mediated effect. In the presence of 6,7-dinitroquinoxaline-2,3-dione and d-2-amino-5-phosphonovaleric acid, 1S,3R-ACPD reversibly depressed directly evoked neocortical IPSPs; however, quisqualic acid (1–10 μM) did not mimic this effect. Analysis of spontaneous PSPs and paired-pulse facilitation indicated a presynaptic locus of action for 1S,3R-ACPD at mGluRs. These findings indicate that a specific mGluR subtype(s) may modulate both excitatory and inhibitory synaptic transmission in the adult rat neocortex via a presynaptic reduction of transmitter release.

[Key words: 1S,3R-ACPD, L-AP4, MCPG, glutamate, metabotropic, neocortex, presynaptic]

L-Glutamic acid is widely accepted as the major excitatory neurotransmitter at many synapses in the mammalian CNS. Based on their signal transduction mechanisms, glutamate receptors can be broadly classified into two general groups, ionotropic and metabotropic. The ionotropic excitatory amino acid (EAA) receptor class includes the NMDA and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors. Ionotropic receptors are generally located postsynaptically, are glutamate-gated, nonspecific cation channel complexes, and mediate fast excitatory synaptic transmission (Collingridge and Lester, 1989; Monaghan et al., 1989; Barnes and Henley, 1992; Nakanishi, 1992). Metabotropic glutamate receptors (mGluRs) are not directly associated with ion fluxes. Instead, they activate second messenger–mediated, biochemical signaling cascades via GTP-binding proteins (G-proteins) (Monaghan et al., 1989; Conn and Desai, 1991; Barnes and Henley, 1992; Baskys, 1992; Nakanishi, 1992; Schoepf, 1993; Schoepf and Conn, 1993).

Molecular characterization of mGluRs has revealed a family of mGluR subtypes coupled to G-proteins (Houamed et al., 1991; Masu et al., 1991; Abe et al., 1992; Tanabe et al., 1992; Nakajima et al., 1993). At present, there are at least seven mGluRs, termed mGluR1–mGluR7 (Houamed et al., 1991; Masu et al., 1991; Abe et al., 1992; Nakanishi, 1992; Tanabe et al., 1992; Nakajima et al., 1993; Saugstad et al., 1993). In situ localization of mRNA coding for the different mGluRs reveals overlapping, but distinct, expression sites, including the neocortex (Abe et al., 1992; Martin et al., 1992; Shigemoto et al., 1992; Tanabe et al., 1992, 1993; Nakajima et al., 1993; Ohishii et al., 1993). The mGluR subtypes also utilize different second-messenger effector systems; for example, mGluR1 increases phosphatidylhydroxynol (PH) hydrolysis and mobilizes intracellular Ca2+; liberates arachidonic acid, and inhibits forskolin-stimulated accumulation of intracellular cAMP (Masu et al., 1991; Aramori and Nakanishi, 1992). mGluR5 only induces PI hydrolysis (Abe et al., 1992), while the other mGluRs appear to inhibit forskolin-stimulated accumulation of intracellular cAMP (Frezeau et al., 1992; Tanabe et al., 1992, 1993; Thomsen et al., 1992; Nakajima et al., 1993; Saugstad et al., 1993). While the different subtypes show varied agonist affinities, the most selective agonist described for the mGluRs is the active isomer of trans-1-aminoctane-1,3-dicarboxylic acid, 1S,3R-ACPD (Schoepf et al., 1990; Conn and Desai, 1991; Baskys, 1992; Nakanishi, 1992; Schoepf, 1993; Schoepf and Conn, 1993).

Electrophysiological studies in a number of brain regions have revealed a wide variety of neuromodulatory actions resulting from mGluR activation. For example, application of glutamate, quisqualic acid, or trans-ACPD hyperpolarizes cerebellar granule cells (Fagni et al., 1991), whereas these mGluR agonists depolarize hippocampal neurons (Stratton et al., 1989, 1990; Chrapak et al., 1990; Desai and Conn, 1991; Desai et al., 1992). Excitatory postsynaptic potentials (EPSPs) are reduced by mGluR activation in the hippocampus (Baskys and Malenka, 1991; Desai et al., 1997). cerebellum (Crepel et al., 1991; Glaum et al., 1992), nucleus of the tractus solitarius (Glaum and Miller, 1992), and the striatum (Lovingier, 1991; Calabresi et al., 1992, 1993; Lovingier et al., 1993). mGluR agonists also reduce inhibitory PSPs (IPSPs) in these brain regions (Desai and Conn, 1991; Calabresi et al., 1992; Desai et al., 1992; Glaum and Miller, 1992). Long-term potentiation can be facilitated by mGluR activation in the hippocampal CA1 region (Anikszeit et al., 1992; Bashir et al., 1993) and in the dorsal lateral septal nucleus (Zheng...
and Gallagher, 1992). mGluR activation can also mediate long-term depression in cerebellar Purkinje cells (Linden et al., 1991). As a result, there have been numerous theories concerning the role(s) of the mGluR subtypes and their subsequent biochemical cascades in normal synaptic transmission (Schoepp et al., 1990; Anwyl, 1991; Conn and Desai, 1991; Baskys, 1992; Nakanishi, 1992; Schoepp, 1993; Schoepp and Conn, 1993) and in pathological states such as neurotoxicity and epilepsy (Schoepp et al., 1990; Conn and Desai, 1991; Nakanishi, 1992; Schoepp, 1993; Schoepp and Conn, 1993).

Despite their marked expression in the neocortex, the role of mGluRs in normal synaptic transmission in this brain region is unclear. Our goal was to examine the effects of mGluR activation on synaptic transmission in the rat frontal neocortex. Conventional intracellular recording techniques were used to record PSPs evoked by intracortical stimulation. Our results show that synaptic transmission is reversibly depressed during mGluR activation, and the data suggest a presynaptic locus of action.

Preliminary reports of some of these data have been published elsewhere (Burke and Hablitz, 1992).

Materials and Methods

Slices of frontal neocortex were obtained from adult Sprague-Dawley rats of both sexes (125-250 gm). Rats were decapitated under ether or ketamine (100 mg/kg) anesthesia, and the brains were quickly dissected out and placed in ice-cold saline for 30-60 sec. The tissue was blocked with a razor blade to separate the hemispheres and to remove the caudal and ventral portions of the brain. The tissue block was attached to a Teflon chuck with cyanoacrylate (Superglue). Four or five coronal slices (500 μm thick) of frontal neocortex were prepared on a Vibroslicer (Campden Instruments), and incubated in a holding chamber at room temperature for at least 2 hr. Single brain slices were transferred to an interface-type recording chamber, and maintained with constant saline perfusion (1 ml/min). The chamber was warmed slowly to the recording temperature of 34 ± 1°C. The extracellular solution consisted of the following (in mM): NaCl, 125; KCl, 3.5; NaH?PO?, 1.25; CaCl?, 2.5; MgSO?, 1.3; NaHCO?, 26; glucose, 10. This solution was continuously aerated with a mixture of 95% O? and 5% CO? to attain a steady state level of oxygenation and to maintain a pH of 7.4.

Conventional intracellular recording electrodes were pulled from filament-containing borosilicate glass tubing (1.5 mm o.d.; A-M Systems, Inc.) and filled with 4 M potassium acetate (adjusted to pH 7.2 with acetic acid). Electrode resistances ranged from 30 to 110 MΩ. Recordings were acquired with the use of an Axoclamp 2-A amplifier (Axon Instruments) in bridge mode. Voltage signals were digitized on line with pclamp 5.2 software (Axon Instruments) or were recorded onto videotape via a Neuro-Corder DR-384 (NeuroData Instruments) to be digitized at a later time. Records were stored on disk and analyzed with pclamp software. Spontaneous PSPs were digitized and subsequently analyzed using Strathclyde Electrophysiological Software (courtesy of J. Dempster, University of Strathclyde, Glasgow). Neuronal membrane potential was monitored continuously on a Gould RS2000 oscillograph (Gould Inc.). A mono- or bipolar stimulating electrode was placed intracortically in layers IV-V slightly lateral to the recording electrode.

After impalement of layer II-III neurons (N = 89 from 70 rats), the resting membrane potential was allowed to stabilize for a minimum of 20 min. Neuronal input resistance was monitored in bridge mode with a -0.4 nA constant amplitude current pulse of 100 nsec duration. All measurements were performed at the original resting membrane potential for each neuron. Constant amplitude current pulses of variable duration were delivered through the stimulating electrode to activate fibers projecting to layer II-III neurons. Postsynaptic potentials (PSPs) in response to increasing stimulus durations (40-180 sec) were evoked at 10-15 sec intervals before, during, and after bath application of putative mGluR agonists and antagonists. In the presence of 6-cyano-7-nitroquinolinicaine-2,3-dione (CNQX) and d1-2-amino-5-phosphonovaleric acid (D-AP5), the effects of mGluR activation on excitatory amino acid antagonist-resistant PSPs were investigated.

The voltage dependence of these directly evoked inhibitory PSPs was determined by injecting constant depolarizing current through the recording electrode. Bridge balance was monitored continuously.

To examine paired pulse facilitation (PPF) of neocortical PSPs, stimulus parameters (i.e., intensity and duration) that evoked primarily excitatory PSPs were used. Interpulse intervals of 10-200 msec were used. Facilitation was calculated as the ratio between the amplitude of the second (test) response and the first (conditioning) response in a pair. During drug application, PPF was tested with the control intensity and with an increased stimulus intensity (to maintain the amplitude of the first PSP at control levels). Control records were compared to those obtained during mGluR activation.

CNQX, D-AP5, L-2-amino-3-phosphonopropionic acid (L-AP3), L-2-amino-4-phosphonobutyric acid (L-AP4), trans-ACPD, 1R,3S-ACPD, 1S,3R-ACPD (R,S)- and (+)-α-methyl-4-carboxyphenylglycine (MCPG) were purchased from Tocris Neuramin; 3,7-dimethyl-1-propargylxanthine (DMPX), from Research Biochemicals, Inc.; and quisqualic acid, from Cambridge Research Biochemicals Limited. These compounds were dissolved in distilled H2O and prepared as 10 mM stock solutions (except MCPG, 1.1 equivalents of 110 mM NaOH and quisqualic acid, 10% ammonium hydroxide). They were then frozen and added in small aliquots to the physiological saline during the experiment. All compounds were applied via the bath, and each neuron served as its own control.

Each trace shown is the average of two responses to a given stimulus. All values are expressed as mean ± SD, except where noted. Statistical analysis consisted of paired and unpaired t tests. A significance level of 0.05 was used.

Results

PSPs evoked during the control period were compared to those evoked during bath application of pharmacological agents. Three points of measure were analyzed: peak PSP amplitude in response to minimum stimulus, peak amplitude to maximum stimulus, and the peak amplitude of the delayed depolarizing response to maximum stimulus. Stimulus-response curves were determined for each measurement. Minimum stimulation (40 sec) produced a purely excitatory PSP, whereas the response to maximal stimulation (180 sec) was an EPSP-IPSP complex. The early component of the compound PSP was predominantly excitatory, while the late depolarizing response was primarily a GABA, receptor-mediated Cl conductance (Connors et al., 1988; Sutor and Hablitz, 1989a, b; Hablitz and Sutor, 1990).

trans-ACPD reduces evoked synaptic transmission in the adult rat neocortex

The evoked PSP to minimum stimulation before and during bath application of 50 μM trans-ACPD is shown in Figure 1A. In this example, only a slight reduction in the peak amplitude was observed (to 96.6% of control). As shown in Figure 1B, however, the response to maximal stimulation was more notably depressed. In this cell, the peak amplitude to maximum stimulation was reduced to 84.8% of control. The late depolarization was similarly reduced to 77.9% of control. No change in resting membrane potential (RMP; -89 mV) was noted in this cell during trans-ACPD application. Neuronal input resistance (Ri) prior to and during drug application was 24.9 MΩ and 27.8 MΩ, respectively. In Figure 1, C and D present input-output curves for the peak amplitudes of the early response and the late depolarization, respectively. These plots show that synaptic potentials were reduced at all but the weakest stimulus strengths. Mean values for these data with 20-50 μM trans-ACPD (N = 8) are presented in Table 1.

1S,3R-ACPD is the active stereoisomer

trans-ACPD is a conformationally restricted analog of the extended form of glutamate, consisting of a 1:1 racemic mixture.
Figure 1. *trans*-ACPD decreases synaptic transmission in the adult neocortex. Intracellular recordings of evoked synaptic activity in a layer II-III neuron are shown in A and B. A, Minimum electrical stimulation produces a small PSP that was marginally inhibited by 50 μM *trans*-ACPD (96.6% of control). B, In the same neuron, more intense stimulation produced an EPSP-IPSP complex. IPSP is depolarizing due to the high RMP (-89 mV). Both excitatory and inhibitory components are reduced by *trans*-ACPD (to 84.8% and 77.9% of control, respectively). C and D, Plot of response amplitude as a function of stimulus intensity for the peak and late depolarizing responses, respectively, during control (●) and in the presence of drug (○).

In Figure 2C, the peak PSP amplitude to minimal stimulation was 99.4% of control; the peak response to maximum stimulation was 100.4% of control during 1R,3S-ACPD application, and the late depolarization was 89.7% of control. As shown in Table 1, synaptic and membrane properties varied less than 10% from control values in the presence of 200 μM 1R,3S-ACPD (N = 3).

Dose dependence of 1S,3R-ACPD effects
The dose–response curves for the three measured components of the evoked PSPs are shown in Figure 3. Responses have been normalized to the maximum percentage reduction produced by 200 μM 1S,3R-ACPD (N = 12) for each of the three PSP measurements (Table 1). The EC₅₀ for the mGluR-mediated reduction of the peak response to minimum stimulation by 1S,3R-ACPD was 30.23 μM. The peak response and the late depolarizing response to maximum stimulation had similar EC₅₀ values of 33.57 μM and 32.66 μM, respectively.

Seventeen of 40 cells tested with 5–200 μM 1S,3R-ACPD depolarized slightly during drug application (depolarizations ranged from 1 to 4 mV). Statistically significant differences in RMP and Rₘ were noted only for 100 and 200 μM 1S,3R-ACPD (Table 1). Pooled data from all experiments indicate a statistically significant trend toward depolarization (−88.6 ± 3.1 and −87.7 ± 3.0 mV, control vs drug, respectively) and an increased Rₘ measured at the original RMP (20.7 ± 4.3 and 22.4 ± 4.6 MΩ, control vs drug, respectively) during the application of 1S,3R-ACPD.

*L-AP4 reduces postsynaptic potentials*
L-2-Amino-4-phosphonobutyric acid (L-AP4) has been reported to antagonize some responses mediated by mGluR activation (Schoepp and Johnson, 1988; Zheng and Gallagher, 1992).
Figure 2. 1S,3R-ACPD reversibly reduces synaptic potentials. A, Plot of EPSP amplitude as a function of time. Bath application of 1S,3R-ACPD (100 μM) for the time indicated by the horizontal bar reduced the peak amplitude of evoked PSPs to 47.2% of control. After 27 min of exposure, response amplitude recovered to control levels after removal of ACPD. B, Specimen records of PSPs in a layer II-III neuron. RMP was -89 mV. Evoked PSPs to minimum (left traces) and maximum (right traces) stimulation were reversibly inhibited by 1S,3R-ACPD. C, As in B, but recordings from another neuron; RMP was -88 mV. 1R,3S-ACPD did not reduce synaptic transmission in the adult rat neocortex.

However, L-AP4 also activates a presynaptic glutamate receptor that depresses synaptic transmission (Harris and Cotman, 1983; Cotman et al., 1986; Forsythe and Clements, 1990; Baskys and Malenka, 1991; Rainnie and Shinnick-Gallagher, 1992; Calabresi et al., 1993) via a G-protein–coupled mechanism (Trombley and Westbrook, 1992). This electrophysiologically defined receptor has been termed the AP4 receptor (Collingridge and Lester, 1989; Monaghan et al., 1989). Recently, L-AP4 has been shown to activate the mGluR4 subtype, which is negatively coupled to the cAMP cascade (Thomsen et al., 1992; Tanabe et al., 1993), and it has been suggested that the AP4 receptor and the mGluR4 are the same glutamate receptor subtype (Nakanishi, 1992; Thomsen et al., 1992; Schoepf and Conn, 1993; Tanabe et al., 1993; but see Trombley and Westbrook, 1992).

We wished to determine the effects of bath-applied L-AP4 on synaptic transmission and passive membrane properties in the adult neocortex prior to examining its putative antagonism of mGluRs. Bath application of L-AP4 (200–1000 μM, N = 4) consistently reduced evoked PSPs, but had no effect on Rm or RMP (Table 1). An example of the L-AP4-mediated effect is shown in Figure 4. The minimum response was reduced to 65.7% of control by 200 μM L-AP4 (Fig. 4A). In Figure 4B, the peak response to maximum stimulation was reduced to 71.9%, and the late depolarization to maximum stimulation was similarly reduced to 58.5% of control. The input–output relationships for the early and late PSP measurements are shown in Figure 4, C and D, respectively. Responses were reduced at all but the smallest stimulus strengths. The effects of mGluR agonists on synaptic and membrane properties of neocortical neurons are summarized in Table 1.

Putative mGluR antagonists

Recent studies have indicated that (R,S)-α-methyl-4-carboxyphenylglycine (MCPG), or its active form, (+)-MCPG, is a competitive mGluR antagonist (Bashir et al., 1993; Eaton et al., 1993; Frenguelli et al., 1993; Jane et al., 1993). Bath application of (+)-MCPG (250–1000 μM, N = 6) for 30 min did not affect synaptic transmission in neocortical slices. When 200 μM 1S,3R-ACPD was bath applied to MCPG-treated slices, little antagonism of the mGluR-mediated synaptic depression was observed. PSPs to minimum stimulation were 92.0 ± 22.8% of control amplitude in MCPG; these PSPs were reduced to 56.4 ± 26.9% with ACPD application (Fig. 5A). In the presence of MCPG, the peak PSP amplitude to maximum stimulation was 103.6 ± 11.9% of control. 1S,3R-ACPD reduced these responses to 58.7 ± 13.1% (Fig. 5B). During MCPG application, the late depolarization to maximum stimulation was 102.3 ± 15.6% of control amplitude, and was reduced to 59.4 ± 24.1% (Fig. 5C). These changes were statistically significant. Thus, MCPG does not antagonize mGluR-mediated synaptic depression in the adult neocortex. Effective MCPG concentrations...
Figure 4. L-AP4 depresses synaptic responses in the neocortex. Intracellular recordings of responses to intracortical stimulation were obtained before and during bath application of L-AP4; RMP was -90 mV. A and B, L-AP4 (200 μM) reduced PSPs evoked by minimum (A, to 65.7% of control) and maximum stimulation (B, to 71.9% and 58.5% of control, peak and late response, respectively). C and D, Input-output relationships for peak amplitude and late depolarizing response, respectively, before (●) and during L-AP4 exposure (●). Responses were reduced at all but the smallest stimulus strengths.

were achieved in the slice, because 1S,3R-ACPD-mediated changes in spike accommodation (J. P. Burke and J. J. Hablitz, unpublished observations) were blocked. This suggests that MCPG is an effective antagonist at postsynaptic mGluRs in the adult neocortex, but may be less effective (or inactive) at presynaptic mGluRs (see Frenguelli et al., 1993).

High concentrations of L-2-amino-3-phosphonopropionic acid (L-AP3) have been shown to block mGluR-mediated PI hydrolysis (Schoepp and Johnson, 1989; Houamed et al., 1991; Desai et al., 1992), intracellular Ca²⁺ mobilization (Irving et al., 1990), and increased cAMP accumulation (Winder and Conn, 1993). L-AP3 also has been reported to block some electrophysiological effects of mGluR activation (Izumi et al., 1991; Linden et al., 1991; Zheng and Gallagher, 1992; Sahara and Westbrook, 1993), whereas other studies show no mGluR antagonism by L-AP3 (Stratton et al., 1990; Chrapak and Gähwiler, 1991; Desai et al., 1992; Glaum et al., 1992; Bashir et al., 1993; Lovinger et al., 1993).

We found that bath application of L-AP3 (200-1000 μM, N = 3) was without effect on synaptic transmission in the neocortex. When slices were bathed in L-AP3 for at least 30 min prior to applying 1S,3R-ACPD (100-200 μM), no antagonism of the mGluR-mediated synaptic depression was observed. These concentrations of ACPD were selected because of their reliability in reducing PSP amplitude and because of the general ineffectiveness of lower ACPD concentrations (see Table 1).

Table 1. Agonist effects at mGluRs

| Drug            | RMP (mV) | Rₘ (MΩ) | Minimum* % change | Max early* % change | Max late* % change |
|-----------------|----------|---------|-------------------|---------------------|--------------------|
| trans-ACPD      | 20-50    | Control | Drug              | Control             | Drug               |
|                 |          | 80.1 ± 4.7 | -77.9 ± 6.5*              | -22.8 ± 23.5          | -22.7 ± 14.6*      |
| 1S,3R-ACPD      | 5        | 87.5 ± 4.5 | -87.8 ± 3.9          | 19.5 ± 3.9           | 20.6 ± 4.0         |
|                 | 10       | 91.6 ± 1.5 | -90.0 ± 1.2          | 18.8 ± 2.9           | 20.0 ± 4.5         |
|                 | 50       | 89.0 ± 3.1 | -88.4 ± 3.0          | 23.7 ± 5.3           | 25.2 ± 6.4         |
|                 | 100      | 87.9 ± 2.8 | -86.4 ± 3.2*         | 20.4 ± 3.7           | 21.8 ± 2.6*        |
|                 | 200      | 88.1 ± 30 | -87.3 ± 2.8*         | 20.8 ± 4.8           | 22.9 ± 5.2*        |
| 1R,3S-ACPD      | 200      | 87.7 ± 0.6 | -88.0 ± 2.6          | 29.8 ± 9.2           | 29.0 ± 8.8         |
| L-AP4           | 200-1000 | 84.5 ± 9.1 | -86.0 ± 6.7          | 28.6 ± 9.9           | 28.4 ± 12.2        |

Data are given as mean ± SD. Each neuron served as its own control. All measurements were performed at each neuron’s original resting membrane potential (RMP).

* Significant differences between control and drug conditions (p < 0.05, paired Student’s t test).
PSPs to minimum stimulation were 103.1 ± 8.4% of control amplitude in L-AP3; these PSPs were reduced to 51.8 ± 17.8% with ACPD application (Fig. 5A). In the presence of L-AP3, the peak PSP amplitude to maximum stimulation was 99.0 ± 8.4% of control. 1S,3R-ACPD reduced these responses to 47.1 ± 5.9% (Fig. 5B). During L-AP3 application, the late depolarization to maximum stimulation was 93.1 ± 11.8% of control amplitude; this response was reduced to 26.1 ± 7.0% (Fig. 5C). Differences between PSP amplitudes in the presence of L-AP3 and in the combined presence of L-AP3 and ACPD were significant for responses evoked by both minimum and maximum stimulation. L-AP3 also did not antagonize postsynaptic effects of mGluR activation (Burke and Hablitz, unpublished observations). Thus, L-AP3 is not an effective agonist at neocortical mGluRs mediating electrophysiologic responses.

**Adenosine receptors do not mediate the reduction in synaptic transmission by ACPD**

Activation of hippocampal adenosine receptors has been shown to decrease synaptic transmission via a presynaptic mechanism (Yoon and Rothman, 1991; Prince and Stevens, 1992; Thompson et al., 1992). Recent reports have demonstrated interactions between mGluRs and adrenergic receptors that increase cAMP accumulation in hippocampus (Winder and Conn, 1993) and decrease accumulation in the cerebral cortex (Cartmell et al., 1993). To investigate the possibility that endogenous adenosine might play a role in the effects of mGluR activation in the neocortex, 3,7-dimethyl-1-propargylxanthine (DMPX; 50-100 μM, N = 2) was bath applied at least 30 min prior to 1S,3R-ACPD application. DMPX is a selective A₁ receptor antagonist with an EC₅₀ of 11 ± 4 μM; the EC₅₀ for the A₂ receptor is 45 ± 5 μM (Seale et al., 1988). DMPX did not affect evoked synaptic transmission (data not shown). When 1S,3R-ACPD (200 μM) was bath applied with DMPX, typical reductions in the peak amplitudes of the synaptic responses were observed. PSPs to minimum stimulation were reduced to 48.1 ± 24.6% of control amplitudes (Fig. 5A). The peak amplitude to maximum stimulation was reduced to 40.1 ± 13.2% of control (Fig. 5B), and the late depolarizing response to maximum stimulation was reduced to 35.6 ± 6.4% of control with 1S,3R-ACPD (Fig. 5C). These data indicate that endogenous adenosine does not play a role in the synaptic depression mediated by 1S,3R-ACPD in the neocortex.

**mGluR activation reduces directly evoked IPSPs**

After blocking the excitatory components of evoked PSPs with the excitatory amino acid receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D(-)-2-amino-5-phosphonovaleric acid (D-AP5), we tested the effects of mGluR activation on IPSPs evoked by direct stimulation of interneurons. CNQX (10 μM) blocks the non-NMDA receptor-mediated component, and D-AP5 (20 μM) blocks the NMDA receptor-associated component of neocortical EPSPs (Sutor and Hablitz, 1989a,b; Hablitz and Sutor, 1990). In Figure 6, A and B show control responses and those recorded during bath application of the EAA antagonists, respectively. The resulting PSPs were evoked at the same stimulus intensities used during the control period. PSPs evoked during EAA receptor antagonism reversed at membrane potentials near −75 mV (Fig. 6C), indicating that they were CI⁻-mediated GABAergic IPSPs (Connors et al., 1988; Sutor and Hablitz, 1989a; Hablitz and Sutor, 1990). Bath application of 50 μM 1S,3R-ACPD (Fig. 6D) reduced the peak amplitude of the response to maximum stimulation to 56.4% of control. Results from all cells tested are shown in Figure 6F. The effect of 1S,3R-ACPD on directly evoked IPSPs was reversible upon washing out.

**Figure 5.** Alterations in PSPs following application of putative mGluR antagonists. The effects of MCPG, L-AP3, and DMPX on evoked synaptic transmission were tested. The percentage of control PSP amplitude to minimum stimulation (A), peak amplitude to maximum stimulation (B), and late depolarization to maximum stimulation (C) during antagonist only and antagonist plus 1S,3R-ACPD (100-200 μM) are illustrated. Means ± SEM are displayed; N = 2-6 for each treatment.
Presynaptic locus of action

Changes in synaptic efficacy producing a change in PSP amplitude might arise from two sources: (1) changes in the sensitivity of the postsynaptic site and/or (2) changes in the output of the presynaptic terminals. We investigated whether 1S,3R-ACPD blocked spontaneous PSPs at concentrations that blocked evoked PSPs by about 50%. In contrast to the effects on evoked synaptic transmission, 1S,3R-ACPD (50-200 μM, N = 12) did not decrease the amplitude of spontaneous PSPs. Under control conditions, mean spontaneous PSP amplitude was 1.49 ± 0.13 mV (mean ± SEM); in the presence of 1S,3R-ACPD, the mean amplitude essentially was unchanged, 1.43 ± 0.08 mV (Fig. 6A). Consistent with a presynaptic site of action, drug application increased the mean spontaneous PSP interval from 17.54 ± 7.14 sec (mean ± SEM) to 42.41 ± 11.73 sec (Fig. 6B). These observations suggest that 1S,3R-ACPD induces a presynaptic decrease in neurotransmitter release. The effects of 1S,3R-ACPD were reversible upon washing.

Paired pulse facilitation (PPF) is a phenomena seen at many chemical synapses and is generally thought to reflect a presynaptic enhancement in neurotransmitter release (Zucker, 1989). Manipulations that reduce transmitter release have been shown to increase PPF (Mallart and Martin, 1967; Katz and Miledi, 1968; Harris and Cotman, 1983). In the following set of experiments, a stimulus strength that evoked an EPSP was used. Interpulse intervals of 10-200 msec were tested, and the ratio of the second response amplitude to that of the first response was calculated. Control records were compared to those obtained during ACPD application. Exposure to 100 μM 1S,3R-ACPD reduced the synaptic response by 39.5 ± 20.9% (N = 4), and enhanced PPF at all interstimulus intervals (data not shown). Because there is normally a greater percentage facilitation at smaller PSP amplitudes, PPF also was tested with an increased stimulus intensity that matched the conditioning PSP amplitude to that recorded during the control period. Under these conditions, facilitation remained increased at all interpulse intervals (data not shown). Statistically significant results were observed for intervals between 10 and 120 msec.

Discussion

Previous studies have shown that ACPD reduces synaptic transmission in a variety of brain regions (Baskys and Malenka, 1991; Henley, 1992), we blocked ionotropic receptor subtypes with 10 μM CNQX and 20 μM D-AP5. Under these conditions, quisqualic acid (1-10 μM, N = 4) did not alter directly evoked IPSPs. As shown in Figure 6E, peak IPSP amplitude was not reduced by 10 μM quisqualic acid application (97.6% of control). The mean effect of quisqualic acid on evoked IPSP amplitude is shown graphically next to that of 1S,3R-ACPD (Fig. 6F). No significant difference in IPSP amplitude or RMP was observed (-85.3 ± 2.1 vs -84.3 ± 0.5 mV).

Figure 6. Directly evoked IPSPs in neocortical neurons are reduced by 1S,3R-ACPD, but not by quisqualic acid. A and B, PSPs evoked under control conditions and in the presence of EAA receptor antagonists, respectively. The same stimulus intensities were used in A and B. RMP was -86 mV. C, Specimen records of PSPs obtained at different membrane potentials. Membrane potential was changed by injecting steady depolarizing current through the recording pipette; RMP was -88 mV. The apparent reversal potential near -75 mV indicates that these are Cl- mediated IPSPs (see Results). D, 1S,3R-ACPD (50 μM) reduced peak IPSP amplitude to 56.4% of control; RMP was -86 mV. E, In a different neuron (RMP = -87 mV), application of quisqualic acid (10 μM) did not depress peak IPSP amplitude. F, Means ± SEM percentage of control for 1S,3R-ACPD (50-100 μM, N = 6) versus quisqualic acid (1-10 μM, N = 4) are illustrated.
Crepel et al., 1991; Desai and Conn, 1991; Lovinger, 1991; Calabresi et al., 1992, 1993; Desai et al., 1992; Glaum and Miller, 1992; Glaum et al., 1992; Rainnie and Shinnick-Gallagher, 1992; Lovinger et al., 1993; Swartz et al., 1993). Our data demonstrate that metabotropic glutamate receptor activation in the adult rat neocortex produces a dose-dependent, reversible depression of synaptic transmission. Furthermore, these results indicate a presynaptic site of action for agonists working at mGluRs, presumably acting to decrease neurotransmitter release. Pharmacological profiles additionally suggest mGluR subtype-specific actions in the neocortex.

**Locus of mGluR action**

Activation of neocortical mGluRs by 1S,3R-ACPD reduced the three measured components of evoked PSPs. Decreased synaptic transmission mediated by 1S,3R-ACPD could occur as the result of antagonism of a postsynaptic glutamate receptor, activation of a postsynaptic receptor that reduces $R_m$ of the cell, or the activation of a presynaptic receptor that reduces neurotransmitter release. The pharmacological profile of 1S,3R-ACPD makes antagonism of postsynaptic glutamate receptors unlikely. This compound is specific for mGluRs with no appreciable ionotropic glutamate receptor activity even at mM concentrations (Schoepp et al., 1991a; Sacaan and Schoepp, 1992). Additionally, under conditions of EAA receptor antagonism, directly evoked IPSPs were reversibly inhibited by 1S,3R-ACPD. This observation is consistent with the finding that mGluR responses are not antagonized by ionotropic EAA-receptor antagonists (Palmer et al., 1988; Reecens et al., 1988; Schoepp and Johnson, 1988), and indicates that 1S,3R-ACPD does not reduce synaptic transmission in the neocortex by antagonizing ionotropic glutamate receptors.

In most neurons examined, application of 1S,3R-ACPD did not decrease neuronal input resistance. To the contrary, higher concentration of 1S,3R-ACPD tended to increase $R_m$ in layer II-III neurons. Pooled data indicate a statistically significant trend toward a slight increase in $R_m$ and a small depolarization with 1S,3R-ACPD application. However, 1S,3R-ACPD-mediated depression of evoked synaptic transmission was observed in neurons that showed slight changes in $R_m$ and RMP, as well as those showing no change. Thus, it is unlikely that the changes in evoked PSPs are the result of alterations in passive membrane properties secondary to the activation of a postsynaptic receptor.

Our findings support the hypothesis that 1S,3R-ACPD acts via the activation of presynaptic mGluRs. The reduction in both ionotropic glutamate receptor-mediated transmission and GABAergic transmission by 1S,3R-ACPD strongly suggests a presynaptic locus of action, with mGluR receptors present on both excitatory and inhibitory terminals. Direct evidence that 1S,3R-ACPD decreases synaptic transmission via a presynaptic action was obtained from investigations of spontaneous PSPs and paired-pulse facilitation of neocortical PSPs. At concentrations that reduced evoked PSPs by about 50%, 1S,3R-ACPD did not reduce spontaneous PSP amplitude. However, the frequency of spontaneous PSPs was markedly decreased. These results are consistent with a presynaptic locus of action: 1S,3R-ACPD reduces transmitter release without changing postsynaptic responsiveness to transmitter. A postsynaptic receptor blocker would reduce responses produced by both stimulus-evoked and spontaneous release of transmitter. In the neocortex, 1S,3R-ACPD enhances facilitation associated with paired-pulse stimulation. The observed increase in facilitation is an expected consequence of decreasing transmitter release (Mallart and Martin, 1968). It is presumed that PPF is influenced by presynaptic Ca$^{2+}$ ion availability (Zucker, 1989). Manipulations of extracellular Ca$^{2+}$ concentrations that affect synaptic transmission has been shown to inversely affect PPF (Katz and Miledi, 1968; Mallart and Martin, 1968; Creager et al., 1980). Harris and Cotman (1983) suggest that this mechanism may be responsible for the actions of L-AP4 in the hippocampus. Recent reports indicate that mGluR activation by a variety of agonists can depress Ca$^{2+}$ currents (Lester and Jahr, 1990; Sayer et al., 1992; Trombley and Westbrook, 1992; Swartz et al., 1993). The effects of ACPD and L-AP4 on synaptic transmission and PPF are consistent with a presynaptic decrease in a Ca$^{2+}$ current involved in neocortical transmitter release (see Sayer et al., 1992, Swartz et al., 1993).

**Pharmacological profile for neocortical mGluRs**

The activity of trans-ACP4 is reportedly restricted to the 1S,3R-stereoisomer (Irving et al., 1990; Schoepp et al., 1991; but see Manzoni et al., 1992). In the adult rat neocortex, 1S,3R-ACPD reversibly reduced synaptic transmission in a dose-dependent manner. In the presence of 200 $\mu$M 1R,3S-ACPD (a concentration of 1S,3R-ACPD that reduced PSPs by >50%), all evoked responses were within 90% of control values. Thus, the selective, presynaptic mGluR properties of trans-ACP4 reside in the 1S,3R-stereo conformation.

L-AP4 has been touted as a putative mGluR antagonist (Schoepp and Johnson, 1988; Zheng and Gallagher, 1992), as well as an agonist at presynaptic glutamate autoreceptors (Harris and Cotman, 1983; Cotman et al., 1986; Forsythe and Clements, 1990; Baskys and Malenka, 1991; Rainnie and Shinnick-Gallagher, 1992; Trombley and Westbrooki, 1992; Calabresi et al., 1993). Our findings that L-AP4 reduces synaptic transmission...
in the neocortex are consistent with the latter reports and, further, the suggestion that a mGluR subtype may be the electrophysiologically defined AP4 receptor (Nakanishi, 1992; Thompson et al., 1992; Schoepp and Conn, 1993; I anate et al., 1993). However, two types of presynaptic glutamate receptors may exist, one that is sensitive to L-AP4 and ACPD, and another one that is sensitive to AC PD, but not to L-AP4 (Loving er al., 1993). Additionally, Sahara and Westbrook (1993) observed that AC PD and L-AP4 modulate Ca" currents via distinct G-protein-coupled receptors in hippocampal cultures. Such complexity could arise from regional variations in expression of mGluR subtypes and differential coupling to effector mechanisms. It is possible that more than one subtype is responsible for the presynaptic effects of I5,3R-ACPD, which would explain the lower efficacy of L-AP4 versus I5,3R-ACPD in the rat neocortex. Further investigations with subtype-specific agonists and antagonists will be required to elucidate the identity and the specific functions of the mGluR subtypes.

While L-glutamic acid is the most likely endogenous agonist for the mGluRs, quisqualic acid is a potent agonist for a number of mGluR subtypes (Nakanishi, 1992; Schoepp, 1993; Schoepp and Conn, 1993). We evaluated the possible activation of mGluRs by quisqualic acid in slices in which ionotropic EAA receptors were blocked. Under these conditions, we failed to observe statistically significant reductions in IPSP amplitude. However, quisqualic acid did enhance Rm in all cells tested. It is possible that quisqualic acid does not activate the mGluR(s) responsible for presynaptically reducing PSP amplitude, but that this compound is a potent agonist for mGluRs located postsynaptically and therefore capable of increasing Rm in neocortical neurons in layers II-III.

Our studies with the putative mGluR antagonists MCPG and L-AP3 indicate that both compounds were poor antagonists of I5,3R-ACPD-mediated synaptic depression in the neocortical slice. MCPG, however, was effective at blocking the direct postsynaptic effects of I5,3R-ACPD (Burke and Hablitz, unpublished observations). Thus, MCPG may be an effective antagonist at postsynaptic mGluRs in the adult neocortex, but is less effective at presynaptically located mGluRs (see Franceschetti et al., 1993). The lack of pre- or postsynaptic antagonism by L-AP3 is consistent with most electrophysiological findings (Stratton et al., 1990; Charpak and Gähwiler, 1991; Desai et al., 1992; Glum et al., 1992; Hu and Storm, 1992; Loving er et al., 1993). This suggests that the mGluRs that are responsible for the reduction of synaptic transmission in the neocortex are not the same mGluRs that are L-AP3 sensitive and linked to PI hydrolysis (Schoepp and Johnson, 1989; Nouaud et al., 1991; Desai et al., 1992), intracellular Ca" mobilization (Irving et al., 1990), or increased cAMP accumulation (Winder and Conn, 1993). However, our studies do not identify the mGluR-mediated second messenger system(s) involved in this form of synaptic modulation.

The functional role of the mGluRs in the adult neocortex is still unclear. It is obvious from this study that activation of these receptors has the functional consequences of presynaptic inhibition and postsynaptic excitation. Under "normal" conditions, these receptors may function at a low level to increase the signal to noise levels of synaptic responses. In pathologic situations, excessive mGluR activation may result, causing either excitatory or inhibitory consequences, depending on the receptor subtypes that are activated.

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