Evidence for Functional Metabotropic Glutamate Receptors in the Dorsal Cochlear Nucleus

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Molitor, Scott C. and Paul B. Manis. Evidence for functional metabotropic glutamate receptors in the dorsal cochlear nucleus. J. Neurophysiol. 77: 1889–1905, 1997. The parallel fibers (PFs) of the dorsal cochlear nucleus (DCN) molecular layer use glutamate as a neurotransmitter. Although metabotropic glutamate receptors (mGluRs) have been identified on cells postsynaptic to the PFs, little is known about the effects of mGluR activation in PF synaptic transmission in the DCN. To investigate these effects, PF-evoked field potentials were recorded from the DCN in guinea pig brain stem slice preparations. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated components of the field response were reversibly depressed by bathing the slice in the mGluR agonists (±)-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD) or (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD]. A similar depression was produced by the mGluR1/5 agonist (RS)-3,5-dihydroxyphenylglycine, but not by the mGluR2/3 agonist (2S,1’5S)-2-(carboxycyclopropyl)glycine or by the mGluR4 agonist (+)-2-amino-4-phosphonobutyric acid. In addition to the AMPA component, an N-methyl-d-aspartate (NMDA) receptor-dependent component of the field potentials could be identified when the slices were bathed in a low magnesium solution. Under these conditions, the ACPD-induced depression of the AMPA component did not completely recover, whereas the depression of the NMDA component usually recovered and potentiated in some slices. Intracellular recordings of PF-evoked responses were obtained to ascertain which neuronal populations were affected by mGluR activation. Activation of mGluRs produced a reversible depression of PF-evoked responses in cartwheel cells that was not accompanied by any changes in paired-pulse facilitation. The PF-evoked responses recorded from pyramidal cells were unaffected by mGluR activation. Both cell types exhibited a reversible depolarization during (1S,3R)-ACPD application. Subsequent experiments explored the involvement of protein kinases in mediating the effects of mGluRs. The protein kinase C (PKC) activator phorbol-12,13-diacetate partially inhibited the mGluR-mediated depression of the field response; however, the PKC inhibitor 2-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)-maleimide or the protein kinase A inhibitor N-[2-{[(p-bromocinnamyl)-amino ethyl]-5-isouquinolinesulfonamide had little effect on the actions of (1S,3R)-ACPD. These results demonstrate that functional mGluRs are present at PF synapses and are capable of modulating PF synaptic transmission in the DCN.

INTRODUCTION

The small-caliber unmyelinated axons of cochlear nucleus granule cells, called parallel fibers (PFs), provide the primary excitatory innervation of the molecular layer of the dorsal cochlear nucleus (DCN). These fibers appear to use glutamate as their primary neurotransmitter (Greenamyre et al. 1984; Manis 1989; Manis and Molitor 1996; Osen et al. 1995; Schwartz 1981). Glutamate can act on at least three different classes of receptors in this system. A fast excitatory component is mediated by non- N-methyl-d-aspartate (non-NMDA) glutamatergic receptors (Manis 1989), of which a significant proportion are α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) selective (Bettler and Muller 1995; Hunter et al. 1993). A slow component is mediated by NMDA receptors that are present on both pyramidal and cartwheel cells (Bilik et al. 1995; Golding and Oertel 1994; Manis and Molitor 1996; Sato et al. 1995; Watanabe et al. 1994). These two receptor systems directly mediate the opening of ion channels and lead to depolarizing synaptic potentials in the cells postsynaptic to the PFs.

A third set of receptors present on some DCN cells are the metabotropic glutamate receptors (mGluRs), which operate through the stimulation or inhibition of G proteins. Recent studies have identified several different mGluR subtypes in the DCN (Petralia et al. 1996; Rubio et al. 1996; Shigemoto et al. 1995; Wright et al. 1996). These receptors can couple the binding of glutamate to the activation of any one of several different second-messenger cascades, depending on the receptor subtype (Nakanishi 1994; Pin and Duvoisin 1995; Schoepp and Conn 1993). Interestingly, neurons of the outer two layers of the DCN have been shown to express a variety of second-messenger system components, including protein kinase C (PKC), adenylate cyclase, and inositol 1,4,5-trisphosphate (IP3) receptors, at relatively high levels (Garcia et al. 1995; Mignery et al. 1989; Ryugo et al. 1995; Saito et al. 1988; Worley et al. 1986). Thus it appears that the mGluRs may be positioned to influence the activity of these second-messenger systems.

In the present study, we investigated the effects of acute activation of mGluRs on PF-mediated synaptic transmission in the DCN, as well as the pharmacology of the mGluRs within the DCN. Activation of mGluRs has been shown to influence neuronal responses to AMPA and NMDA (Aniksztejn et al. 1991; Fitzjohn et al. 1996; Glaucom et al. 1992; Harvey et al. 1993; Kelso et al. 1992), so both receptor types were investigated in these experiments. In addition, intracellular recordings were obtained to ascertain the effects of mGluR activation on both the cartwheel and pyramidal cells, which comprise the major cell populations in the outer layers of the DCN that receive PF input (Hackney et al. 1990; Lorente de Nó 1981; Osen 1969). Because it is known...
that mGluRs exert their effects through at least two different metabolic pathways (Nakanishi 1994; Pin and Duvoisin 1995; Schoepp and Conn 1993), an attempt was made to elucidate the mechanisms by which mGluR activation affects the AMPA receptor-mediated component of synaptic transmission within the DCN.

Methods

Preparation of slices

The response of DCN neurons during pharmacological stimulation of mGluRs was assessed with extracellular and intracellular recording techniques with the use of the guinea pig DCN slice preparation (Manis 1989, 1990). Although both rat and guinea pig DCN have been shown to be immunoreactive for mGluRs (Petralia et al. 1996; Rubio et al. 1996; Shigemoto et al. 1992; Wright et al. 1996), the guinea pig was selected for these experiments because its nucleus is more distinctly laminated than that of the rat, which results in larger field potentials and facilitates electrode placement. Briefly, young pigmented guinea pigs weighing 150–300 g were anesthetized with pentobarbital sodium (35–40 mg/kg) and decapitated, and the brain stems were quickly removed and the brain stems and were then placed into an incubation chamber containing a sodium bicarbonate buffer that was continually perfused with 95% O2-5% CO2 at 34°C. Slices were incubated for ≥1 h before being transferred to the recording chamber.

Electrophysiological recordings

Both extracellular field potential and intracellular current-clamp recordings were used to assess the response of cochlear nucleus neuronal populations to acute metabotropic receptor activation. Field potentials were recorded from layer 2 with the use of 10- to 15-MΩ electrodes filled with 250 mM NaCl and 10 mM HEPES, pH adjusted to 7.3 with NaOH. Field recordings were amplified (×200) and low-pass filtered at 2 kHz (Intra 767, World Precision Instruments). In some field potential experiments, a second electrode was placed in layer 1 to monitor the amplitude of the compound action potential of the PFs. Intracellular recordings were obtained from cells in either layer 1 or 2 with the use of 40- to 100-MΩ electrodes filled with 2.7 M KAc and 5 mM KCl. Penetrations were typically obtained with a brief current pulse or oscillation; cells were identified by their characteristic responses to depolarizing current injection (Manis 1990; Manis et al. 1994). Intracellular recordings were amplified (×10) and low-pass filtered at 3 kHz (8700 Cell Explorer, Dagan Instruments), and a constant hyperpolarizing current was typically applied (<1.5 nA) to minimize the contamination of excitatory postsynaptic potentials (EPSPs) with active conductances. Input resistance and bridge balance were continuously monitored with short pulses of hyperpolarizing current. Initial bridge balance errors were corrected online; subsequent changes in bridge balance during the course of recording were calculated and corrected for off-line (see Data analysis). Both extracellular and intracellular recordings were digitized at 5–20 kHz with a 12-bit A/D converter (Digidata 1200, Axon Instruments). Additional gain was obtained by adjusting the range of the analog input within the A/D converter (±1.25 V for extracellular and ±2.5 V for intracellular recordings). PFs were stimulated with the use of a 250-μm coaxial electrode placed on the ependymal surface ≥500 μm away from the recording electrode. Recordings were obtained with the stimulation electrode located either dorsal or ventral to the recording electrode, and no apparent differences were observed in responses elicited with either orientation. This arrangement results in responses generated primarily by activating elements residing within the molecular layer (Manis 1989), and should minimize contamination by pathways residing deeper in the nucleus, such as auditory nerve afferents. Care was taken to avoid stimulation of the dorsal acoustic stria, which would result in antidromic stimulation of pyramidal cells (Adams and Warr 1976). Constant monopolar or bipolar current pulses (250–600 μA, 100 μs in duration) were delivered alone or in pairs separated by 40 ms and presented at 20- or 30-s intervals. During extracellular recordings, the stimulus intensity was adjusted so that the peak of the P32 wave (see Fig. 1) in layer 2 was ~50–75% of maximal amplitude. Active conductances prevented an accurate determination of maximal EPSP amplitude during intracellular recordings, so the stimulus intensity was adjusted to maximize EPSPs while attempting to maintain the cell below the threshold for active spike generation throughout the course of the experiment.

Solutions

The dissection solution used during the slice preparation contained (in mM) 138 NaCl, 5 KCl, 1.25 KH2PO4, 10 glucose, 0.2 CaCl2, 4 MgSO4, and 10 HEPES, pH adjusted to 7.35 with ~5 mM NaOH. The incubation and recording solutions contained (in mM) 130 NaCl, 3 KCl, 1.25 KH2PO4, 20 NaHCO3, 10 glucose, 2.5 CaCl2, and 1.3 MgSO4. In addition, 2–5 μM strychnine and 10 μM picrotoxin were added to the recording solution to suppress inhibitory postsynaptic potentials. The low magnesium solution used in the experiments examining the modulation of NMDA receptors had the same composition as the incubation and recording solutions, with the exception that this solution contained 20 μM MgSO4 and 50 μM glycine to facilitate the NMDA receptor-mediated component of the synaptic responses (Johnson and Ascher 1987; Nowak et al. 1984). Slices were incubated in this low Mg2+ solution supplemented with 25 μM D-aminophosphonovalerate (D-APV) for ≥1 h before recording commenced. When the slices were transferred to the recording chamber, the D-APV was quickly washed out, revealing the NMDA receptor-dependent component of the field potential (see Fig. 7A). This procedure was adopted because preliminary experiments showed that the time course of washing the normal magnesium (1.3 mM) from the slice was slow and variable, leading to a drifting baseline amplitude of the NMDA receptor-dependent component that interfered with clearly demonstrating the effects of acute mGluR activation. The solution flow was maintained at 2–4 ml/min and the temperature of the chamber was 32°C throughout recording.

The following drugs were applied alone or in combination: (±)-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD); (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD]; (1R,3S)-1-aminocyclopentane-1,3-dicarboxylic acid [(1R,3S)-ACPD]; (RS)-3,5-dihydroxyphenylglycine (DHPG); (28.1’S,2’S)-2-(carboxycyclopropyl)glycine (1-CCG-I); 1-(+)-2-amino-4-phosphonobutyric acid (1-AP4); DL-2-amino-3-phosphonopropionic acid (DL-AP3); (S)-α-methyl-4-carboxyphenylglycine (MCPG); D-APV; phorbol-12,13-diacetate (PDAC); 2-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)-maleimide (bisindolylmaleimide); and N-[2-(p-bromocinnamyl) amino] ethyl]-5-isouquinolinesulfonamide (H-89). For the experiments investigating the effects of mGluR activation during PKC blockade with bisindolylmaleimide, slices were incubated for ≥1 h in the standard incubation solution supplemented with 1 μM bisindolylmaleimide. Previous experiments had demonstrated that bisindolylmaleimide was most effective in blocking PDAC-induced activation of PKC in the DCN if slices had been pretreated with bisindolylmaleimide before application of PDAC (Francis and Manis 1995). Similarly, slices were pretreated for ≥1 h in the
FIG. 1. Averaged field potential responses to parallel fiber (PF) stimulation in the dorsal cochlear nucleus (DCN). Thin lines: average responses over a 5-min period just before drug application. Thick lines: averaged responses over a 5-min period, starting 5 min after the bathing solution was changed to one containing (±)- 1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD; 50 μM). A: field potential responses from layer 1. The magnitude of the afferent fiber volley is determined by subtracting the minimum of the N1 waveform from an average of the maxima of the P1 and P2 waveforms. The postsynaptic current sink, N2, is also labeled. trans-ACPD does not affect the afferent fiber volley, but does reduce the postsynaptic current sink. Calibration bar: 4 ms, 0.75 mV. B: field potential responses from layer 2. The magnitude of the postsynaptic population spike is determined by subtracting the N2 minimum from the P2 maximum; the magnitude of the field excitatory postsynaptic potential (EPSP) is calculated by subtracting the prestimulus baseline from the P3 maximum. The afferent fiber volley, N12, is also labeled. trans-ACPD significantly reduces both the postsynaptic population spike and the field EPSP without affecting the afferent fiber volley. Calibration bar: 4 ms, 0.25 mV. Stimulus artifacts have been blanked for clarity.

standard incubation solution containing 1 μM H-89 for experiments investigating the effects of mGluR activation during protein kinase A (PKA) blockade. All drugs were either added directly into the recording solution, or were diluted from concentrated stock solutions in distilled water or dimethyl sulfoxide. Bisindolylmaleimide, PDAc, and H-89 were purchased from Calbiochem; L-APV was purchased from Cambridge Research Biochemicals and Research Biochemicals; trans-ACPD and DL-AP3 were purchased from Research Biochemicals; and (1S,3R)-ACPD, (1R,3S)-ACPD, DHPG, L-CCG-I, L-AP4, and MCPG were purchased from Tocris Cookson. All remaining compounds were purchased from Sigma or Aldrich.

Data analysis

Stimulation of the PFs results in characteristic field potentials recorded in layers 1 and 2 of the DCN (Manis 1989). In layer 1, the negative-going afferent compound action potential is the largest component of the response (Fig. 1A). This afferent fiber volley is labeled N1 (the subscript identifies the layer in which the component is recorded). This wave is followed by a small, slower negative wave, corresponding to the postsynaptic current sink (N2). The magnitude of the afferent fiber volley is found by subtracting the minimum of the N1 from the average of the maxima of the positive-going waves (P1 and P2) on either side of N1. In layer 2, the response consists of the afferent fiber volley (N12), the population spike (N22), and the postsynaptic current source (P32) or the field EPSP (Fig. 1B). The magnitude of the population spike was found by subtracting the minimum of the N22 from the maximum of the P22; the magnitude of the field EPSP was calculated by subtracting the prestimulus baseline from the maximum of the P32. In low magnesium containing solutions, there was an additional component present, the P4 (Manis and Molitor 1996). The magnitude of this wave was found by computing the area under the waveform between 30 and 50 ms after the stimulus (see Fig. 7A). Magnitudes of the various waveform features are calculated for each slice throughout the experiment, normalized relative to the average values obtained during the control period, and averaged across all slices. Both paired and unpaired Student’s t-tests were employed for statistical comparisons; data samples were obtained from individual slices as the average magnitude across the last 5 min of a drug application normalized to the average magnitude during control period. Average values are presented as means ± SE unless otherwise noted. Field potential data are presented from 90 slices obtained from 26 animals.

The analysis of the intracellularly recorded EPSPs was similar to that of extracellularly recorded field EPSPs. In some cells, PF stimulation produced action potentials superimposed on EPSPs during the acute activation of mGluRs with (1S,3R)-ACPD. To eliminate action potentials from the EPSP analysis, a threshold was set (typically 40–50 mV above the prestimulus baseline) and any responses that exceeded this threshold were discarded. An analysis of the effects of acute mGluR activation on the intracellular resting potential was also conducted. Time-dependent variations in passive cellular properties over the course of many experiments affected the measurement of the intracellular resting potential. At the initiation of recordings, both the bridge balance correction and capacity compensation were set and not altered throughout the duration of the recording. To monitor any changes in the bridge balance, 40-ms pulses of hyperpolarizing current were applied after each stimulus pair, and the resulting charging curves were fit with a biexponential function with the use of a simplex algorithm. The magnitude of any discontinuity between the prepulse baseline and the biexponential fit at the start of the current pulse was assumed to be a bridge balance error. This error was used to correct the measured resting potential if any holding current was applied. In addition, cells typically exhibited a steady hyperpolarizing drift in resting potential over the course of an experiment, which may reflect a drift in junction potentials or intracellular potassium loading from the high potassium electrodes. To correct for this, a linear regression of the resting potential over the control period was performed and the resulting slope was subtracted from the entire resting potential time course. The resulting resting potential time courses were then normalized as an absolute deviation from the average values measured during the control period, and averaged across slices. Some resting potential time courses were excluded from these averages if they exhibited large discontinuities or fluctuations (>2 mV) during the control period. Intracellular recording data are presented from 16 slices obtained from 11 animals.
FIG. 2. Summary of the responses of the various PF-evoked field potential components to trans-ACPD (50 μM). Time courses are normalized to the control period and averaged across slices. Thick bar above each plot shows when the valves were changed to admit the bathing solution containing trans-ACPD. A: trans-ACPD does not significantly affect the afferent fiber volley (97.5 ± 2.9% of control, mean ± SE; N = 3) recorded in layer 1. B and C: trans-ACPD depresses the postsynaptic population spike (62.6 ± 7.0% of control, N = 8) and the field EPSP (67.1 ± 5.8% of control, N = 11) recorded in layer 2. The effects of trans-ACPD on the postsynaptic components of the response are rapid and reversible. Points in each plot are the mean data from N = 3–11 slices. Error bars: means ± SE. Dashed lines in each panel: average of the normalized control trace. Across all experiments, changes in paired-pulse facilitation during exposure to 50 μM trans-ACPD are quite variable and not significant at the P = 0.05 level (Fig. 3, B and C). Thus it is not clear from these results whether presynaptic mGluRs are responsible for the field EPSP depression produced by trans-ACPD.

mGluR pharmacology

Various agonists and antagonists were utilized to investigate the pharmacology of mGluRs within the DCN. Application of 20 μM (1S,3R)-ACPD, a stereoisomer of trans-ACPD, produces a reversible depression of the field EPSP (Fig. 4A) similar to that observed with the application of 50 μM trans-ACPD. The other stereoisomer of trans-ACPD, (1R,3S)-ACPD (100 μM), had no significant effects on the field EPSP in six slices (Fig. 4B). A slow, irreversible depression of the field EPSP occurred during the application of 100 μM (1R,3S)-ACPD in a seventh slice. Because it is not known whether this depression was due to the drug application or to other circumstances, this slice was excluded from this analysis. A dose-response analysis of the effects of (1S,3R)-ACPD on the PF-evoked field EPSP was conducted (Fig. 4C). A simplex fit of the field EPSP magnitudes during the application of 0.1–100 μM (1S,3R)-ACPD showed a maximal depression to 55.7 ± 7.6% of control values (mean ± 95% confidence interval), and that the half-maximal concentration of (1S,3R)-ACPD was 7.3 ± 3.9 μM.

Further experiments were performed to characterize the mGluR subtypes that mediate the reversible field EPSP depression during the application of (1S,3R)-ACPD. Application of DHPG (100 μM), a specific agonist of the mGluR1/5 subtypes (Gereau and Conn 1995b; Ito et al. 1992), results in a depression of the field EPSP (Fig. 5A) that is similar in magnitude to the depression produced by 20 μM (1S,3R)-ACPD. This depression is not completely reversible and only recovers to 86.2 ± 4.9% of the control EPSP magnitude 20 min after application of DHPG. The mGluR2/3 agonist l-CCG-I (2 μM) (Hayashi et al. 1992) produces a small reversible depression of the field EPSPs (Fig. 5B); however, the magnitude of this depression is significantly smaller than that produced by 20 μM (1S,3R)-ACPD. Similarly, the mGluR4/6/7/8 agonist l-AP4 (20 μM) (Duvoisin et al. 1995; Nakajima et al. 1993; Okamoto et al. 1994; Saugstad et al. 1994; Tanabe et al. 1993) produces a reversible depres-
FIG. 3. Effects of trans-ACPD on paired-pulse facilitation. A: averaged traces showing the differential depression of the 1st and 2nd field EPSPs (R1 and R2) elicited by a pair of stimuli (S1 and S2) before and after the application of trans-ACPD (50 μM). Thin line: average response over a 5-min period just before drug application. Thick line: average response over a 5-min period, starting 5 min after changing the bathing solution to one containing trans-ACPD. B: trans-ACPD produces a reversible depression of the field EPSP elicited by the 2nd stimulus that is similar to that elicited by the 1st stimulus. Thick line obscured by the data shows a 5-point boxcar average of the data from Fig. 2 for direct comparison with the averaged field EPSPs elicited by the 1st stimulus. C: trans-ACPD produces a slight reversible increase in the paired-pulse facilitation, which is defined as R2/R1. Individual points represent the averaged absolute facilitation across slices and are not normalized to facilitation values from the control period. Error bars: means ± SE. Dashed line: average facilitation during the control period (142.9 ± 3.0%, N = 11); the average facilitation during trans-ACPD application increases slightly (165.5 ± 12.2%). Direct comparison of the average facilitation values from individual slices before and during trans-ACPD application shows that the facilitation change is not significant at the 5% level (t = 2.16, P = 0.06).

sion of the field EPSP that is also significantly smaller than that produced by 20 μM (1S,3R)-ACPD (Fig. 5C). A higher concentration of l-AP4 (200 μM) produced no additional depression of the field EPSP (94.3 ± 3.4% of control, N = 3; data not shown). l-CCG-I was not tested at higher concentrations, because this compound will activate mGluR1s and mGluR4s at higher concentrations (Hayashi et al. 1992).

Reported antagonists of mGluRs were applied in conjunction with mGluR agonists and the subsequent effects on the field EPSPs were observed. DL-AP3 (100 μM), previously reported to be an antagonist of ibotenate-stimulated phosphatidylinositol turnover at submillimolar concentrations (Schoepp and Johnson 1989), was ineffective in antagonizing the depression of the field EPSP when applied with 50 μM trans-ACPD (Fig. 6A). It should be noted that higher concentrations of both DL-AP3 and L-AP3 produced an irreversible depression of the field EPSP in the absence of trans-ACPD (data not shown), and therefore could not be used to antagonize the depression induced by trans-ACPD. MCPG (250 μM), a phenylglycine derivative that has been reported to be an antagonist of the mGluR1 and mGluR2 subtypes at submillimolar concentrations (Hayashi et al. 1994; Thomsen et al. 1994), partially blocked the depression of the field EPSP when applied with 20 μM (1S,3R)-ACPD (Fig. 6B). The application of 250 μM MCPG alone had no effects on the magnitude of the field EPSP (data not shown), and higher concentrations were not tried.

Modulation of NMDA receptor-dependent responses

The data presented to this point are from recordings of PF-evoked field EPSPs in solutions that contain 1.3 mM Mg2+, resulting in responses that are primarily mediated by non-NMDA ionotropic receptors (Manis 1989). We subsequently examined the modulation of NMDA receptor-dependent responses by bathing the slices in a low magnesium solution, which reveals a slow NMDA receptor-dependent component in the field response, or the P4 (Fig. 7A, thin trace) (see also Manis and Molitor 1996). This component is blocked by 25 μM d-APV (Fig. 7A, thick trace) or by 1.3 mM Mg2+ (Fig. 7A, thin trace obscured by thick trace). Initial experiments showed that the time course of washing magnesium from the slice was slow and variable. To ensure that ambient magnesium concentrations remained at equilibrium throughout the duration of the recording, subsequent
FIG. 4. Effects of the stereoisomers \((1S,3R)-1\text{-aminocyclopentane-1,3-dicarboxylic acid} \ (1S,3R)-\text{ACPD})\) and \((1R,3S)-1\text{-aminocyclopentane-1,3-dicarboxylic acid} \ (1R,3S)-\text{ACPD})\) on the field EPSP magnitude. \(A\) and \(B\): \((1S,3R)-\text{ACPD} \ (20 \mu M)\) reversibly depresses the field EPSP \(63.5 \pm 3.6\% , N = 7)\), whereas \((1R,3S)-\text{ACPD} \ (100 \mu M)\) has no significant effects \((96.3 \pm 2.1\% , N = 6)\). The effects of trans-\text{ACPD} and \((1S,3R)-\text{ACPD}\) on the field EPSP are not significantly different (comparing data from Figs. 2C and 4A, \(t = 0.46, P = 0.65\)). \((1R,3S)-\text{ACPD}\) did produce an irreversible depression of the field EPSP magnitude in 1 slice and was not included in the average shown in \(B\). \(C\): dose dependence of the field EPSP depression on \((1S,3R)-\text{ACPD}\). Individual points represent the magnitude of the field EPSP over the last 5 min of a 10-min drug application period, normalized to the control period, and then averaged across 5–7 slices. Error bars: mean ± SE. Line: best fit of the equation

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\% \text{control} = (100 - \frac{\text{IC}_{50}^c}{\text{IC}_{50}^t + [(1S,3R)-\text{ACPD}]})
\]

where \(I_{\text{max}}\) is the maximal inhibition of the field EPSP (%), \(\text{IC}_{50}^c\) is the half-maximal concentration \((\mu M)\), and \(n\) is the Hill coefficient. The best fit values and their corresponding 95% confidence intervals were determined by a simplex algorithm to be \(I_{\text{max}} = 44.3 \pm 7.6\%, \text{IC}_{50} = 7.3 \pm 3.9 \mu M,\) and \(n = 1.0 \pm 0.4\).

experiments were performed by incubating slices in a low magnesium solution supplemented with 25 \(\mu M\) \(d\)-APV for \(\approx 1\) h before recording. The \(d\)-APV was subsequently washed out for \(\approx 10\) min before the initiation of recordings. In these experiments, a 20-min application of 20 \(\mu M\) \((1S,3R)-\text{ACPD}\) results in a depression of the non-NMDA \((P_3, \text{Fig. 7B})\) and of the NMDA \((P_4, \text{Fig. 7C})\) receptor-mediated components of the field response. There is no significant difference between the depression of the \(P_3\) and the \(P_4\) components during the last 5 min of a 20-min exposure to \((1S,3R)-\text{ACPD} \ (t = 0.92, P = 0.38, N = 12)\). A 10-min exposure to \(m\text{GluR}\) agonists was used in previous experiments; a 20-min exposure to \((1S,3R)-\text{ACPD}\) was used in these experiments because the \(P_4\) component did not reach a steady state during the first 10 min of \((1S,3R)-\text{ACPD}\) application in some slices. Under these conditions, an irreversible depression of the \(P_3\) component is observed up to 40 min after ACPD application (Fig. 7B) that is not seen in the \(P_4\) component (Fig. 7C), resulting in a significant difference between the \(P_3\) and \(P_4\) magnitudes during the last 5 min of the 40-min washout period following \((1S,3R)-\text{ACPD}\) application \((t = 3.25, P = 0.008, N = 12)\). A similar sustained depression was seen after the application of DHPG (Fig. 5A), but it is not clear whether the sustained depression observed in these experiments is due to the prolonged exposure to \((1S,3R)-\text{ACPD}\) or to the combined acti-
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Although the majority of slices experienced a depression of both the fast and slow field EPSP components during (1S,3R)-ACPD application, two lines of evidence suggested that the modulation of both components by mGluRs is not the same. First, the application of 20 μM (1S,3R)-ACPD resulted in a depression of the P3 2 component and a potentiation of the P4 2 component in one slice (Fig. 8A and highest point in Fig. 8C), whereas acute mGluR activation resulted in a small depression of the P3 2 component and a large depression of the P4 2 component in another slice (Fig. 8B and lowest point in Fig. 8C). The remainder of the slices exhibited a depression of both components that was either the same or larger for the P3 2 component (Fig. 8C). Second, the acute activation of mGluRs has a differential long-term effect on the non-NMDA and NMDA receptor-mediated components of the PF-evoked response. As noted previously, the differential long-term effects are primarily due to a sustained depression of the P3 2 component (Fig. 7B) that is not typically observed with the P4 2 component (Fig. 7C). Further examination reveals a range of long-term behaviors for the P4 2 component following ACPD application. Although a few slices exhibit a sustained depression of the P4 2 component, the majority of slices exhibit a complete reversal, and in some cases a sustained potentiation of this component (Fig. 8D). This is in contrast to the P3 2 component; the majority of slices exhibit a sustained depression and in no cases was a sustained potentiation of this component observed. The data in Fig. 8, C and D, also demonstrate that the variability observed in the averages shown in Fig. 7, B and C, is primarily due to variations between slices rather than variations between responses from an individual slice. Therefore, in some slices, the non-NMDA and NMDA components do not appear to be modulated in a similar fashion during and after the acute activation of mGluRs.

Neuronal populations possessing mGluRs

Intracellular current-clamp recordings were performed to assess which neuronal populations postsynaptic to PFs responded to acute mGluR activation. Previous studies have

FIG. 5. Effects of the metabotropic glutamate receptor (mGluR) subtype-specific agonists (RS)-3,5-dihydroxyphenylglycine (DHPG), (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (l-CCG-I), and L(+)-2-amino-4-phosphonobutyric acid (L-AP4) on the magnitude of the field EPSP. A: DHPG (100 μM) produces a depression of the field EPSP (67.6 ± 3.2% of control, N = 5) that is not significantly different from the depression produced by (1S,3R)-ACPD (comparing data from Figs. 4A and 5A, t = 0.80, P = 0.44). Unlike (1S,3R)-ACPD, DHPG produces a depression that is not completely reversible, and returns to 86.2 ± 4.9% of control values 15–20 min after return to the control solution. B: l-CCG-I (2 μM) has a small reversible effect on the field EPSP (93.5 ± 1.0% of control, N = 6) that is significantly different from that produced by (1S,3R)-ACPD (comparing data from Figs. 4A and 5B, t = 7.38, P < 0.001). C: L-AP4 (20 μM) also produces a small reversible depression of the EPSP (91.6 ± 2.9% of control, N = 7) that is significantly different from that produced by (1S,3R)-ACPD (comparing data from Figs. 4A and 5C, t = 6.02, P < 0.001).

FIG. 6. Effects of the mGluR antagonists dl-2-amino-3-phosphonopropionic acid (dl-AP3) and (S)-α-methyl-4-carboxyphenylglycine (MCPG) on mGluR activation. A: dl-AP3 (100 μM) does not significantly antagonize the effects of trans-ACPD (73.9 ± 3.0% of control, N = 6; comparing data from Figs. 2C and 6A, t = 0.82, P = 0.42). Thick line obscured by the data shows a 5-point boxcar average of the data from Fig. 2C for direct comparison. B: MCPG (250 μM) partially antagonizes the field EPSP depression produced by (1S,3R)-ACPD (87.0 ± 6.9% of control, N = 5; comparing data from Figs. 4A and 6B, t = 3.26, P = 0.009). Thick line shows a 5-point boxcar average of the data from Fig. 4A for direct comparison.
shown that the majority of intracellular recordings from the superficial layers of the guinea pig DCN are obtained from either the cartwheel or pyramidal cell populations, and that these two cell types can be distinguished by their characteristic responses to depolarizing current injection (Manis et al. 1994). The application of 20 μM (1S,3R)-ACPD depresses the PF-evoked EPSPs recorded from cartwheel cells (Fig. 9A). Five of six cartwheel cells exhibited a reversible depression of the first (Fig. 9B) and second (Fig. 9C) EPSPs in response to a pair of stimuli separated by 40 ms. The magnitude of this depression is similar to that seen in the field EPSPs during the application of 20 μM (1S,3R)-ACPD. In contrast to the extracellular field EPSPs recorded from some slices, the paired-pulse facilitation of EPSPs recorded from cartwheel cells is unaffected by (1S,3R)-ACPD (20 μM D-APV). In contrast to the extracellular field EPSPs (Fig. 10, A–C) or on the paired-pulse facilitation (Fig. 10D). Despite the differential modulation of EPSPs among the two cell types, all cartwheel and pyramidal cells respond to (1S,3R)-ACPD with a reversible depolarization (Fig. 11). It is not obvious whether any consistent changes in the input resistance accompanied this depolarization. The input resistance typically fluctuated throughout the duration of the recordings and in some cells showed irreversible changes that did not appear to be a result of the drug application (data not shown).

**Second-messenger involvement in mGluR modulation of EPSPs**

The activation of mGluRs can increase the activity of phospholipase C or decrease the activity of adenylate cyclase depending on the mGluR subtype (Pin and Duvoisin 1995; Schoepf and Conn 1993) and thereby can activate PKC through the production of diacylglycerol or inhibit PKA through the reduction of adenosine 3′,5′-cyclic monophos-
FIG. 8. Further comparison of the effects of (1S,3R)-ACPD on the non-NMDA and NMDA receptor-mediated components of the field response. A and B: example time courses of the normalized $P_{42}/P_{32}$ ratio for 2 individual slices demonstrating the variability of mGluR activation on these components. C: plot of the normalized $P_{32}$ magnitudes vs. the normalized $P_{42}$ magnitudes averaged over the last 5 min of a 20-min application of (1S,3R)-ACPD for the 12 slices from Fig. 7, B and C. With the exception of the slices shown in A and B, the activation of mGluRs depresses both the components of the field response, and this depression is either the same for both components or greater for the $P_{32}$ component. D: plot of the normalized $P_{32}$ magnitudes vs. the normalized $P_{42}$ magnitudes averaged over the period 35–40 min after return to the control solution for the 12 slices from Fig. 7, B and C. Of the 12 slices, 3 show a significant irreversible depression of the $P_{42}$ component; the remaining 9 show a complete reversal of this depression, and, in 5 of these 9, a sustained potentiation relative to the control values. In contrast, 8 of the 12 slices show a significant irreversible depression of the $P_{32}$ component and none show any sustained potentiation of this component relative to control values. Dashed line provides a reference for identical changes in $P_{32}$ and $P_{42}$ magnitudes in C and D. Error bars: means ± SE over the 5-min period from which the averages were obtained from individual slices. Horizontal error bars: means ± SE of the $P_{32}$ averages. Vertical error bars: mean ± SE of the $P_{42}$ averages. Error bars are only displayed if they extend beyond the size of the symbols plotted.

Discussion

These experiments demonstrate that activation of mGluRs depresses PF-mediated synaptic transmission in the DCN. A depression of synaptically evoked responses following the application of the mGluR agonists trans-ACPD or (1S,3R)-ACPD (Palmer et al. 1989; Schoepp et al. 1990) has been
observed in various regions of the brain, including the brain stem (Glaum and Miller 1992), cerebellum (Crepel et al. 1991), hippocampus (Baskys and Malenka 1991b), and striatum (Lovinger 1991). Synaptic responses are also depressed by the mGluR agonist L-AP4 (Schoepp and Johnson 1988; Tanabe et al. 1993) in the hippocampus (Koerner and Cotman 1981), spinal cord (Davies and Watkins 1982), and prepyriform cortex (Hearn et al. 1986). Our results indicate that one subtype of ACPD-sensitive mGluRs produces the depression of PF-mediated synaptic transmission in the DCN, and that this depression is specific to only one of the neuronal cell types postsynaptic to the PFs. These mGluRs may play a specific role in modulating PF synaptic transmission in this nucleus.

**Pharmacology of mGluRs in the DCN**

The depression of PF-evoked responses produced by (1S,3R)-ACPDP is mediated by the mGluR1/5 subtypes. At least eight different mGluRs have been cloned and are expressed throughout the mammalian nervous system, and these are classified into three separate groups on the basis of their sequence homology and pharmacology (Pin and Duvoisin 1995): group I, which includes mGluR1 and 5 (Abe et al. 1992; Masu et al. 1991); group II, which includes mGluR2 and 3 (Tanabe et al. 1992); and group III, which includes mGluR4, 6, 7, and 8 (Duvoisin et al. 1995; Nakajima et al. 1993; Okamoto et al. 1994; Saugstad et al. 1994; Tanabe et al. 1992). Application of DHPG, at a concentra-
FIG. 10. Effects of mGluR activation on EPSPs recorded from simple spiking DCN neurons. A: averaged EPSPs in response to a pair of stimuli before (thin trace) and during (thick trace) the application of (1S,3R)-ACPD (20 μM). Inset: response of this cell to 100-ms current pulses (±1.6 nA), which is characteristic of simple spiking neurons in the DCN. Calibration bar: 20 ms and 5 mV for the averaged EPSPs, or 50 ms and 80 mV for the responses to current injection. B and C: (1S,3R)-ACPD produces no significant changes in EPSP magnitudes in response to either the 1st (103.6 ± 6.6% of control, N = 10) or the 2nd stimulus (97.1 ± 4.4% of control, N = 7) of a stimulus pair. Individual points represent the EPSP magnitudes recorded from 7 ± 10 simple spiking cells, normalized to the control period and averaged across cells. Error bars: means ± SE. Note that 3 simple spiking cells began spiking in response to the 2nd stimulus during (1S,3R)-ACPD application and were excluded from the averages shown in C and D. D: (1S,3R)-ACPD does not affect the paired-pulse facilitation of the EPSPs recorded from simple spiking cells. Individual points represent the averaged absolute facilitation across cells and are not normalized to facilitation values from the control period. Error bars: means ± SE. Dashed line: average facilitation during the control period (232.5 ± 13.5%, N = 7); the average facilitation during the last 5 min of a 10-min (1S,3R)-ACPD application (236.1 ± 9.4%) does not change significantly (t = 0.42, P = 0.69). Recordings from 3 of the 10 cells shown in B and from 2 of the 7 cells shown in C and D were terminated after application of (1S,3R)-ACPD, before the end of the 40-min period shown in these panels.
FIG. 11. Effects of acute mGluR activation on the resting potential of both complex and simple spiking DCN neurons. Resting potentials were obtained from the prestimulus baseline voltage, normalized as the deviation from the resting potentials obtained during the control period, and averaged across slices. Bridge balance errors were corrected if holding currents were applied (see METHODS). A and B: (1S,3R)-ACPD (20 μM) produces a reversible depolarization in both complex spiking cells (3.3 ± 0.4 mV, N = 5) and simple spiking cells (6.5 ± 2.1 mV, N = 6). Recordings from 3 of the 5 complex spiking cells in A and from 2 of the 6 simple spiking cells in B were terminated after (1S,3R)-ACPD application, before the end of the 40-min period shown in these panels. Discontinuities in the time course of the mean or mean ± SE reflect points at which these recordings were terminated.

A moderate amount of L-AP4 (20 μM) produced a small reversible depression in some slices; however, this depression was significantly smaller than that produced by (1S,3R)-ACPD. In the hippocampus, micromolar amounts of L-AP4 can substantially depress synaptic transmission at the perforant path/dentate granule cell (Koerner and Cotman 1981) and mossy fiber/CA3 pyramidal cell (Cotman et al. 1986) synapses; but submillimolar amounts are needed to produce a strong depression at the Schaffer collateral/CA1 pyramidal cell synapses (Baskys and Malenka 1991a; Gereau and Conn 1995a). Higher concentrations of L-AP4 (200 μM) did not produce any additional depression of PF-evoked field EPSPs in the DCN. Therefore the majority of the mGluR-mediated synaptic depression is mediated through the receptor subtypes sensitive to (1S,3R)-ACPD rather than those sensitive to L-AP4.

Cellular localization of functional mGluRs

It is likely that the depression of PF-evoked responses by mGluR agonists is mediated by mGluR1α receptors in the dendritic spines of cartwheel cells. Affinity-purified polyclonal antibodies to mGluR1α intensely label the dendritic spines of cartwheel cells (Petralia et al. 1996; Wright et al. 1996). This is consistent with our results, which show that the depression of PF-evoked responses is predominantly mediated by the mGluR1/5 subtypes, and that this depression is observed only in recordings from cartwheel cells. It is unclear why one cartwheel cell experienced a slight potentiation of EPSP magnitude during exposure to (1S,3R)-ACPD; it is possible that a subgroup of cartwheel cells may not respond to mGluR activation in a similar fashion. In addition to cartwheel cell dendrites, antibodies to mGluR1α label pyramidal cells (Petralia et al. 1996), but it is not clear from this study whether significant levels of mGluR1α are found near PF synapses. Our experiments do not address the func-

FIG. 12. Involvement of protein kinase C (PKC) and protein kinase A (PKA) in mediating the effects of mGluR activation on PF-evoked extracellular field responses. A: (1S,3R)-ACPD (20 μM) produces a reversible depression of the field EPSPs (82.7 ± 5.2% of control, N = 5) in slices pretreated with phorbol-12,13-diacetate (PDAc, 2 μM); however, this depression is significantly reduced compared with the depression produced without PDAc (comparing data from Figs. 4A and 12A, t = 3.15, P = 0.01). B: (1S,3R)-ACPD reversibly depresses the field EPSPs (71.3 ± 2.7% of control, N = 5) in slices pretreated with 2-[(3-dimethylamino- propyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)-maleimide (bisindolylmaleimide, 1 μM), and this depression is similar to that produced without bisindolylmaleimide (comparing data from Figs. 4A and 12B, t = 1.59, P = 0.14). C: (1S,3R)-ACPD reversibly depresses the field EPSPs (74.9 ± 4.6% of control, N = 5) in slices pretreated with N-[2-((p-bromocinnamyl)amino)ethyl]-5-isquinolinesulfonamide (H-89, 1 μM), and this depression is not statistically different on the 5% level from that produced without H-89 (comparing data from Figs. 4A and 12C, t = 1.98, P = 0.08). Thick lines in each panel show a 5-point boxcar average from the data in Fig. 4A for direct comparison. Data in A are from slices exposed to PDAc for ≥30 min before the application of (1S,3R)-ACPD. Data in B and C are from slices incubated in bisindolylmaleimide or H-89 for ≥1 h before the initiation of recording and continuously superfused with solutions containing bisindolylmaleimide or H-89 throughout the duration of the recording.
tional significance of the high levels of mGluR1α expression found in unipolar brush cell dendrites (Wright et al. 1996). The size, location, and synaptic circuitry of these cells (Floris et al. 1994; Mognaini et al. 1994) make it unlikely that they significantly contribute to the extracellular field responses, or that they are included in the intracellular recordings of responses to PF stimulation. The mGluR2/3 subtypes are present in the basal dendrites of pyramidal cells (Rubio et al. 1996), and are also present in small- to medium-sized cells residing in deeper layers of the DCN, which are likely to be unipolar brush cells (Petralia et al. 1996). Because PF synapses are only found within the most superficial layers of the DCN (Hackney et al. 1990; Lorente de Nó 1981; Osen 1969), the mGluR2/3 subtypes do not appear to be positioned to modulate PF-evoked responses. No significant staining is observed with antibodies to mGluR5 (Petralia et al. 1996), and no immunocytochemical studies have addressed the presence of the remaining mGluR subtypes in the DCN.

In contrast to the cartwheel cells, pyramidal cells possess functional mGluRs that do not modulate PF-evoked responses. Despite the differential effects of mGluR activation on PF-mediated EPSPs in cartwheel and pyramidal cells, both cell types respond to (1S,3R)-ACPD with a reversible depolarization. This depolarization does not appear to be due to nonspecific synaptic activation; all inhibitory synaptic transmission was blocked and no spontaneous EPSPs were observed during the application of (1S,3R)-ACPD. It is possible that this depolarization is due to the direct modulation of voltage-sensitive conductances; activation of mGluRs is known to affect both calcium (Lester and Jahr 1990) and potassium conductances (Charpak et al. 1990). In the hippocampus, an mGluR-mediated closure of potassium channels is responsible for this depolarization (Harata et al. 1996; Shirasaki et al. 1994). In contrast, a similar depolarization in cerebellar Purkinje cells results from an increase in the activity of an electrogenic Na⁺/Ca²⁺ exchanger activated by mGluR-mediated intracellular calcium mobilization (Linden et al. 1994; Staub et al. 1992). The differential localization of mGluR subtypes in cartwheel and pyramidal cells leads to the interesting possibility that this depolarization is mediated by different mechanisms in these two cell types: an mGluR1-induced intracellular calcium release leading to increased Na⁺/Ca²⁺ exchanger activity in the dendrites of cartwheel cells, and an mGluR2/3-induced closure of potassium channels in the basal dendrites of pyramidal cells.

Activation of mGluRs produces a variety of effects in other regions of the auditory brain stem. Prolonged tetanic stimulation of excitatory afferents produces a substantial mGluR-mediated depolarization in the gerbil lateral superior olive (Kotak and Sanes 1994); this depolarization could be mediated by mechanisms similar to the small depolarization seen in both pyramidal and cartwheel cells in the present study. In the chick nucleus magnocellularis, the avian homologue of the mammalian anterior ventral cochlear nucleus, mGluR activation results in the mobilization of intracellular calcium (Zirpel et al. 1995) and the inhibition of an L-type voltage-sensitive calcium conductance (Lachica et al. 1995). In the medial nucleus of the trapezoid body (MNTB) of young rats, it was shown that group III mGluRs presynaptically modulate synaptic transmission (Barnes-Davies and Forsythe 1995), presumably via a reduction in calcium entry through presynaptic P/Q-type voltage-sensitive calcium conductance (Takahashi et al. 1996). Although our experiments do not rule out presynaptic effects, it is clear that the depression of PF-evoked responses in DCN cartwheel cells is mediated by group I mGluRs. Thus, in the auditory system, it seems likely that different modulatory mechanisms at presynaptic and postsynaptic sites can be initiated by a variety of mGluR subtypes.

**Synaptic localization of mGluR modulation of PF-evoked responses**

Our evidence suggests that at least part of the synaptic depression at PF/cartwheel cell synapses is initiated by the postsynaptic action of mGluRs on the cartwheel cells. A similar mGluR-mediated depression of synaptic responses is attributed to a presynaptic reduction of transmitter release in regions such as the hippocampus (Baskys and Malenka 1991a), cerebellum (Glaum et al. 1992), striatum (Lovingier et al. 1991, and MNTB (Barnes-Davies and Forsythe 1995). Three observations support the hypothesis that the mGluR-mediated depression of synaptic responses is mediated by a postsynaptic mechanism in the DCN. First, the pharmacology of the depression of PF-evoked responses and the localization of this depression to cartwheel cells suggests that the mGluR1α receptors expressed in cartwheel cell dendritic spines are responsible for this depression. Despite the intense labeling of cartwheel cell dendritic spines by antibodies to mGluR1α, no labeling of presynaptic terminals at PF synapses has been observed (Petralia et al. 1996; Wright et al. 1996). Therefore any presynaptic depression would have to be mediated either by an unidentified group I mGluR on presynaptic terminals or by retrograde communication across cartwheel cell/PF synapses. Second, following mGluR activation in low magnesium, there is a sustained depression of the AMPA component that is not usually observed in the NMDA component, and in some cases there is a potentiation of the NMDA component. It is possible that this differential modulation of AMPA and NMDA components could be produced by changes in glutamate release, assuming specific patterns of transmitter release and receptor occupancy. However, a more likely explanation involves a postsynaptic site of action, such as a decrease in postsynaptic AMPA receptor sensitivity in the absence of any presynaptic changes or an increase in postsynaptic NMDA receptor sensitivity accompanied by a reduction in presynaptic glutamate release. The latter is known to occur in the hippocampus, where a selective potentiation of NMDA responses has been observed (Aniksztejn et al. 1991) that is mediated by group I mGluRs (Fitzjohn et al. 1996). Third, the lack of any changes in the paired-pulse facilitation during the mGluR-mediated depression of PF-evoked EPSPs during intracellular recordings from cartwheel cells also supports a postsynaptic site of action. Changes in paired-pulse facilitation usually accompany presynaptically mediated alterations in synaptic transmission (e.g., Harris and Cotman 1985). This appears to be the case in hippocampal CA1 pyramidal cells, where an increase in paired-pulse facilitation accompanies the mGluR-mediated depression of Schaffer collateral-evoked EPSCs (Baskys and Malenka 1991b), but not in the MNTB, where no changes...

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in paired-pulse facilitation accompany the depression of synaptic responses by presynaptic group III mGluRs (Barnes-Davies and Forsythe 1995). The paired-pulse facilitation of extracellular field EPSPs increased in some slices during the application of trans-ACPD even though the facilitation of intracellular EPSPs did not change. It is possible that this difference originates from the stimulus conditions: the extracellular field EPSPs were evoked by stimulus levels that may have resulted in contamination of PF-evoked responses by active conductances. Concomitant alterations in active conductances by mGluR activation could then result in paired-pulse facilitation changes that would not be observed under conditions that minimize active conductances. Although our results could be explained solely by a postsynaptic site of action, we cannot rule out that mGluR activation ultimately includes a modulation of presynaptic events.

Mechanism of mGluR modulation of PF responses

The effects of mGluR activation on PF-mediated synaptic transmission do not appear to be mediated through PKA or PKC. Although the PKC activator PDAc partially blunts the effects of (1S,3R)-ACPD, the PKC inhibitor bisindolylmaleimide has no effect on the mGluR-mediated field EPSP depression. If PKC is partially responsible for mediating the synaptic depression, bisindolylmaleimide should also attenuate the effects of (1S,3R)-ACPD in a similar fashion. These results suggest that the synaptic depression produced by mGluRs is not due to PKC but that PKC is able to modulate this depression when activated separately. PKC activation by phorbol esters is known to modulate mGluR-mediated PKC activation in striatal cultures (Manzoni et al. 1990) and mGluR-mediated depression of voltage-sensitive calcium conductances in acutely isolated hippocampal CA3 pyramidal neurons (Schwartz and Bean 1992; Schwartz et al. 1993). The mGluR-mediated synaptic depression does not appear to be mediated by PKA, because the PKA inhibitor H-89 did not significantly affect the depression produced by (1S,3R)-ACPD. However, it is not clear how effective H-89 is as a PKA inhibitor in this preparation, because it did not prevent the forskolin-induced field EPSP potentiation in one of four slices. In addition, H-89 was unable to prevent the depression of the field response produced by adenosine in all three slices tested, although adenosine may act through pathways other than adenylylate cyclase (Olsson and Pearson 1990). Because the mGluR subtypes (mGluR1/5) that mediate the depression of PF synaptic responses have been found to activate phospholipase C rather than to inhibit adenylylate cyclase (Abe et al. 1992; Masu et al. 1991), it is reasonable to assume that cAMP and PKA are not involved in the mGluR-mediated synaptic depression.

One possible pathway for effecting responses to mGluR activation could be calcium release from intracellular stores. IP3 receptors are present at high levels in the superficial DCN, especially in the dendrites of cartwheel cells (Ryugo et al. 1995). However, in cartwheel cells, IP3 receptors are present in the dendritic shafts, whereas the mGluR1α receptors are found in the dendritic spines (Wright et al. 1996). This observation creates two potential problems with the hypothesis that IP3 is the effector for an mGluR-mediated synaptic depression in these cells. First, the level of mGluR activation would have to be sufficient to produce enough IP3 to activate the distal IP3 receptors. Little is known about the diffusion of IP3 within the cytosol and the degradation or uptake of this second messenger within cellular compartments. Second, even if IP3 receptors were activated by mGluRs, the resulting increase in cytosolic calcium would most likely not propagate into the dendritic spines because of the compartmentalization and rapid buffering of cytosolic calcium (Gamble and Koch 1987; Simon and Llinás 1985).

Role of mGluRs in PF-mediated synaptic transmission

The mGluRs present within DCN neurons may play an important role in modulating synaptic transmission in the superficial DCN. We have investigated the effects of acute mGluR activation on PF-evoked responses; no information is available as to whether mGluRs are activated during normal synaptic transmission. In the nucleus tractus solitarius, only high-frequency stimulation of tractus solitarius afferents elicits responses in the presence of excitatory and inhibitory ionotropic receptor antagonists that resemble responses elicited by (1S,3R)-ACPD (Glaum and Miller 1992). The situation may be similar in the DCN, where the perisynaptic localization of cartwheel cell mGluR1α receptors would presumably require high rates of PF stimulation to generate the glutamate levels necessary to activate these receptors (Wright et al. 1996). If conditions are sufficient to activate mGluRs, a modulation of synaptic and voltage-sensitive conductances or increases in cytosolic calcium could influence the short-term responses of cartwheel cells to PF stimulation. Pyramidal cells also have functional mGluRs; however, it is likely that these receptors would not be directly activated by PF stimulation. These mGluRs may be activated by other synaptic inputs, such as excitatory auditory nerve inputs on the basal dendrites of these cells (Gonzalez et al. 1993; Manis and Brownell 1983). Application of mGluR agonists is known to alter the responses of DCN neurons to sound (Sanes et al. 1996); whether this is due to changes in the response to auditory nerve inputs or to a depolarization of these neurons in response to mGluR activation has not been examined.

The presence of functional mGluRs may produce long-term effects as well. Although the effects of (1S,3R)-ACPD were reversible under normal conditions, a small sustained depression of the AMPA receptor-mediated component was observed following (1S,3R)-ACPD application under conditions that activate NMDA receptors. A similar sustained depression was also observed following DHPG application under normal conditions. Sustained changes in acoustic response properties of DCN neurons following the iontophoresis mGluR agonists have also been observed (Sanes et al. 1996). Our experiments suggest that a weak long-term modulation of PF synaptic transmission can be pharmacologically induced by mGluR activation, but we did not address
the conditions required for this to occur. These results differ from those observed in the dentate gyrus, where a sustained potentiation of both AMPA and NMDA receptor-mediated EPSC components is observed following mGluR activation under normal recording conditions (O’Connor et al. 1994, 1995). Such differences in long-term effects may arise from the coupling to different biochemical pathways, or from a differential subcellular localization of target signaling pathways. Even though mGluR activation alone may not generate long-term changes in synaptic responses, it is required for the initiation of long-term changes in synaptic strength in regions such as the hippocampus (Bashir et al. 1993; Bortolotto et al. 1994) and the cerebellum (Kano and Kato 1987; Linden et al. 1991). If such long-term changes in synaptic strength occur in the DCN, mGlur5 could be involved in the initiation of these changes.

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