

# Modulation of mEPSCs in Olfactory Bulb Mitral Cells by Metabotropic Glutamate Receptors

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**Schoppa, N. E. and G. L. Westbrook.** Modulation of mEPSCs in olfactory bulb mitral cells by metabotropic glutamate receptors. *J. Neurophysiol.* 78: 1468–1475, 1997. Olfactory bulb mitral cells express group I (mGluR1), group II (mGluR2), and group III (mGluR7 and mGluR8) metabotropic glutamate receptors. We examined the role of these mGluRs on excitatory synaptic transmission in cultured mitral cells with the use of whole cell patch-clamp recordings. The effects of group-selective mGluR agonists and antagonists were tested on  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-receptor-mediated miniature excitatory postsynaptic currents (mEPSCs). (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylate (ACPD) or the group-I-selective agonist 3,5-dihydroxyphenylglycine evoked an inward current accompanied by a decrease in membrane conductance, consistent with the previously described closure of potassium channels by group I agonists. The increased cellular excitability was accompanied by an increase in mEPSC frequency in some cells. When calcium entry was blocked by cadmium, ACPD or the group-II-selective agonist 2-(2,3-dicarboxycyclopropyl)-glycine reduced the mEPSC frequency. L-2-amino-4-phosphonobutyric acid (L-AP4), a group-III-selective agonist, caused a similar decrease. The concentration-dependence of L-AP4-mediated inhibition was most consistent with activation of mGluR8. We investigated two possible effector mechanisms for the group III presynaptic receptor. Bath application of forskolin or 3-isobutyl-1-methylxanthine had no effect on mEPSC frequency. Increasing calcium influx by raising extracellular  $K^+$  caused a large increase in the mEPSC frequency but did not enhance L-AP4-mediated inhibition. Thus inhibition of mEPSCs involves a mechanism downstream of calcium entry and appears to be independent of adenosine 3',5'-cyclic monophosphate. Our results indicate that both group II and III receptors can inhibit glutamate release at mitral cell terminals. Although group II/III receptors had a similar effect on mEPSCs, differences in location on nerve terminals and in glutamate sensitivity suggest that each mGluR may have discrete actions on mitral cell activity.

## INTRODUCTION

G protein coupled metabotropic glutamate receptors (mGluRs) cause neuromodulatory effects by a number of effector pathways (Saugstad et al. 1995). The diverse actions of mGluRs can, in part, be attributed to the large number of metabotropic receptor subtypes (Pin and Bockaert 1995). Eight different mGluRs (mGluR1–8) have been cloned, and these have been divided into three groups on the basis of primary amino acid sequence homology, pharmacology, and second-messenger coupling. In expression systems, group I receptors (mGluR1 and 5) are coupled to phosphatidyl inositol hydrolysis. Group II receptors (mGluR2 and 3) and group III receptors (mGluR4, 6, 7, and 8) are negatively coupled to adenylate cyclase. Several patterns have emerged from attempts to match specific sub-

types with physiological effects in neurons. For example, the group I mGluRs are largely postsynaptic (Bause et al. 1993; Martin et al. 1992) and can increase excitability by modulating ion channels (e.g., Charpak et al. 1990; Crépel et al. 1994), whereas the effects of group III mGluRs activated selectively by L-amino-4-phosphonobutyric acid (L-AP4) appear to be exclusively presynaptic (Baskys and Malenka 1991; Forsythe and Clements 1990; Gereau and Conn 1995; Mayer and Westbrook 1987; Salt and Eaton 1995; Trombley and Westbrook 1990). Although most neurons express several mGluRs, the expression of individual mGluRs has been shown to have specific functional implications in some pathways. For example, in the accessory olfactory bulb, activation of mGluR2 in granule cells suppresses inhibitory  $\gamma$ -aminobutyric acid (GABA) transmission to mitral cells (Hayashi et al. 1993). Likewise, in hippocampal pyramidal neurons, mGluR7 is expressed presynaptically at synapses with a distinct subset of mGluR1 $\alpha$ -expressing interneurons, presumably allowing autoregulation to be specific to inputs to these interneurons (Shigemoto et al. 1996a).

mGluRs are richly expressed in the main olfactory bulb. Mitral cells, the principal neuron in the bulb, express four of the eight cloned mGluRs including mGluR1, 2, 7, and 8 (Duvoisin et al. 1995; Masu et al. 1991; Ohishi et al. 1993; Okamoto et al. 1994; Saugstad et al. 1994). With the use of selective antibodies, mGluR1 has been localized to the mitral cell body and to its primary and secondary dendrites (Martin et al. 1992; van den Pol. 1995), whereas mGluR7 and 8 have been localized to mitral cell axon terminals (Kinoshita et al. 1996; Kinzie et al. 1997). The different localization patterns for these mGluRs presumably give them distinct functions, but their physiological effects on synaptic transmission in the bulb remain unclear. It is known that L-AP4 causes presynaptic inhibition (Anson and Collins 1987; Trombley and Westbrook 1990) and also reduces mitral cell calcium currents (Trombley and Westbrook 1992). However, it remains to be determined whether the reduction in calcium currents is responsible for L-AP4-mediated presynaptic inhibition.

We examined the action of different mGluRs on excitatory synaptic transmission in cultured mitral cells. Whole cell patch-clamp recordings were made in the presence of tetrodotoxin and, in some cases, cadmium. Group-selective agonists were tested for their effects on miniature excitatory postsynaptic currents (mEPSCs) mediated by  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Our results indicate that both group II and III receptors inhibit transmitter release from mitral cell axons by a mechanism downstream from calcium entry, whereas group I receptors can enhance mEPSC frequency.

## METHODS

*Cell culture*

Primary dissociated cultures of neurons from the olfactory bulb of newborn rat pups were prepared as described previously (Trombley and Westbrook 1990). Rats were maintained in compliance with a protocol approved by the Oregon Health Science University Animal Care Committee. The cultures contained predominantly mitral/tufted cells and granule cells. All recordings were made from mitral/tufted cells, which have large (20–40  $\mu\text{m}$ ) and pyramidal-shaped somata and thus could be easily discriminated from small (5–10  $\mu\text{m}$ ) bipolar granule cells.

*Electrophysiology*

Whole cell patch current recordings were made on mitral/tufted cells after 12–28 days in culture. Patch pipettes were fabricated from borosilicate glass (TW150F, WPI), and pulled with a conventional two-step puller (Narishige). Pipette resistances were 1–3 M $\Omega$ . After seal formation and patch rupture, the access resistances were generally between 4 and 6 M $\Omega$ ; recordings were rejected if the access resistance was >12 M $\Omega$ . No series resistance compensation was used; membrane potential was not corrected for junction potentials. Current signals recorded with an Axopatch 200A amplifier (Axon Instruments) were filtered at 1 kHz with the use of an eight-pole Bessel filter and digitized at 2 kHz. Data were acquired on an IBM 486 clone with the use of pClamp version 6 (Axon Instruments). All experiments were performed at room temperature (20–24°C).

Patch pipettes were filled with standard intracellular solution containing (in mM) 165 CsCl, 4 MgCl<sub>2</sub>, 1 ethylene glycol-bis( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 2 NaATP, 0.5 sodium guanosine 5'-triphosphate, and 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH adjusted to 7.2 with CsOH. In some experiments, CsCl was replaced with an equimolar amount of KCl or cesium methanesulfonate. The extracellular bath solution contained (in mM) 165 NaCl, 2.5 KCl, 2 CaCl<sub>2</sub>, 10 glucose, and 10 HEPES, pH adjusted to 7.3 with NaOH. Tetrodotoxin (1  $\mu\text{M}$ ), picrotoxin (50  $\mu\text{M}$ ), D(-)-2-amino-5-phosphopentanoic acid (D-AP5, 100  $\mu\text{M}$ ), and magnesium (1 mM) were added to the bath to isolate AMPA-receptor-mediated mEPSCs.

Drug solutions were applied with the use of an array of flow pipes (400  $\mu\text{m}$  id). These were fabricated from coated quartz glass, connected to gravity-fed reservoirs, and positioned ~100–200  $\mu\text{m}$  from the cell body of the neuron. Each flow pipe was controlled by solenoid valves. In some of the recordings, the assembly was moved with a piezoelectric bimorph (Vernitron). Whether the assembly was moved with the bimorph or manually, the solution exchange time for the cell was  $\leq$  2 s, as assayed by changes in the holding current on application of a bath solution with 20 mM KCl. This solution exchange time was adequate for the experiments described.

*Experimental procedure*

After a patch seal and successful whole cell, the cell was held at a holding potential of –50 or –60 mV for the duration of the experiment. To test mGluR agonists or antagonists on mEPSCs, a given drug solution was applied for 30- to 60-s intervals separated by similar duration applications of the control solution. The number of drug applications ranged from two to nine. In some recordings, a gradual decline in the mEPSC frequency was observed over the 8–60 min of whole cell recording time. The frequent changes in the test/control solutions reduced errors in our analysis due to these nonstationarities. Data acquisition typically began 2–5 s after the change of solution. In experiments in which forskolin, 3-isobu-

tyl-1-methylxanthine (IBMX), and dideoxy-forskolin were used, reagents were applied for up to 15 min.

*Data analysis*

Analysis of mEPSCs was performed with the use of Axograph (Axon Instruments) on a MacIntosh computer. This program passes a sliding template with an exponential rise and decay; mEPSCs are detected as minima in the error between the scaled template and the measured current. With the appropriately selected rise and decay time constants for the template, the number of mEPSCs detected with the program was within 10% of that determined by visual examination of the current recordings ( $n = 2$ ). Errors in all measured quantities are given as means  $\pm$  SE. Statistical significance was determined with the use of the paired two-sample Student's *t*-test within Microsoft Excel. Only statistically significant differences are reported as different in the text, unless otherwise indicated.

*Materials*

Drugs were obtained from the following sources: D-AP5, L-AP4, (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylate (ACPD), 3,5-dihydroxyphenylglycine (DHPG), (2S,1'S,2'S)-2-(carboxycyclopropyl)-glycine (L-CCG-I), (S)- $\alpha$ -methyl-4-carboxyphenylglycine (MCPG), and methyl-aminophosphonobutyric acid (MAP-4) from Tocris Cookson; tetrodotoxin, picrotoxin, IBMX, forskolin, and 1,9-dideoxy-forskolin from Sigma. 2-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV) was a kind gift from Drs. P. Jeffrey Conn (Emory University) and H. Shinozaki (Tokyo Metropolitan Hospital).

## RESULTS

*ACPD and DHPG increase mitral cell excitability*

The recorded AMPA-receptor-mediated mEPSCs in mitral cells ranged in amplitude from 5 to 50 pA and had rapid rise times (20–80% peak in 200–400  $\mu\text{s}$ ) and decay times (time constant = 1–4 ms), as reported previously for AMPA-receptor-mediated EPSCs (Collinridge and Lester 1989). Figure 1A illustrates the effect of the relatively non-selective mGluR agonist ACPD. In this cell, and in 8 of 24 total cells, ACPD (100  $\mu\text{M}$ ) caused a marked increase in the mEPSC frequency. The effect could not be quantitated because the very high mEPSC frequency in ACPD precluded determination of the current baseline. The high mEPSC frequency also prohibited a reliable estimate of the mEPSC amplitude in ACPD-affected cells, but no amplitude effect was observed in the subset of cells that failed to respond to ACPD with an mEPSC frequency increase, suggesting that ACPD did not affect the postsynaptic responsiveness of AMPA receptors. As exemplified by Fig. 1A, *bottom trace*, and in 4 of 14 cells, the excitatory effect of ACPD on the mEPSC frequency was mimicked by DHPG (30  $\mu\text{M}$ ), which is a group-I-selective agonist (Schoepp et al. 1994), but not by L-CCG-I (2  $\mu\text{M}$ ), which is selective for group II mGluRs and mGluR8 (Hayashi et al. 1992; Saugstad et al. 1997), or the group-III-selective agonist L-AP4 (100  $\mu\text{M}$ ) (Saugstad et al. 1994; Tanabe et al. 1993). The increased excitability was most likely mediated by mGluR1 because it is the only group I receptor expressed in mitral cells (Abe et al. 1992; Masu et al. 1991).

Activation of group I receptors can increase excitability

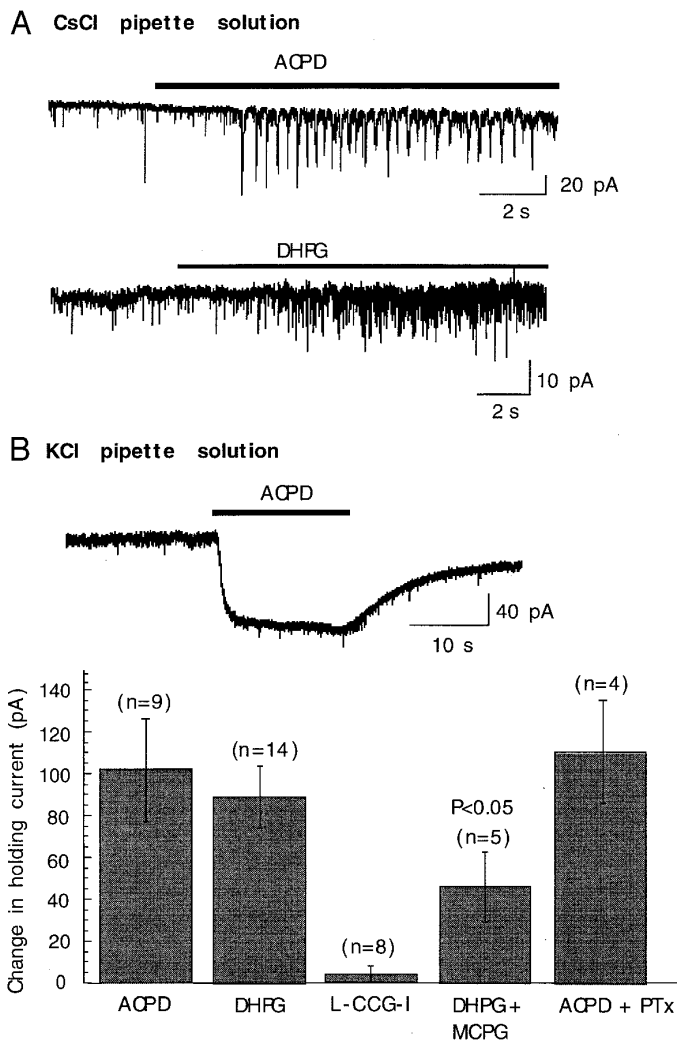


FIG. 1. Activation of a group I metabotropic glutamate receptor (mGluR) increased mitral cell excitability. *A*: in recordings made with CsCl in the pipette, (1*S*,3*R*)-1-amino-cyclopentane-1,3-dicarboxylate (ACPD; 100  $\mu$ M, *top trace*) increased the frequency of miniature excitatory postsynaptic currents (mEPSCs). This effect was mimicked by the group-I-selective agonist 3,5-dihydroxyphenylglycine (DHPG; 30  $\mu$ M, *bottom trace*). *B*: in recordings in which intracellular CsCl was replaced with an equimolar amount of KCl, ACPD also evoked an inward current. Histogram shows that the current was also evoked by DHPG, but not by (2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl)-glycine (L-CCG-I, 2  $\mu$ M). (S)- $\alpha$ -methyl-4-carboxyphenylglycine (MCPG, 500  $\mu$ M) significantly antagonized the effect of DHPG in 5 cells (paired *t*-test). The ACPD-evoked current was not blocked by pretreatment with pertussis toxin (PTx; 250 ng/ml, 20 h). Number of cells for each treatment is indicated at the top of the bars.

in other neurons by closing potassium channels (Charpak et al. 1990). To test this possibility in mitral cells, we replaced the CsCl in the pipette solution with KCl. Under these conditions, both ACPD and DHPG, but not L-CCG-I, caused an increase in inward holding current (Fig. 1*B*; 8 of 10 cells for ACPD and 13 of 14 cells for DHPG). The DHPG-induced current was associated with a  $29 \pm 12\%$  (mean  $\pm$  SE,  $n = 5$ ) increase in cell input resistance (averaging 0.23 and 0.30 G $\Omega$  before and after DHPG), suggesting that the inward current was due to closure of potassium channels. The DHPG-induced current was partially inhibited by MCPG (500  $\mu$ M), which antagonizes glutamate action on

group I and II receptors and mGluR8 (Hayashi et al. 1994; Saugstad et al. 1997). The "postsynaptic" mGluR-induced current presumably also occurs in other mitral/tufted cells that impinge on the recorded cell, leading to depolarization and increased mEPSC frequency. Consistent with this idea, the voltage-gated calcium channel blocker cadmium (100  $\mu$ M) blocked the mEPSC frequency increase evoked by ACPD ( $n = 10$ ; Fig. 2*A*) or DHPG ( $n = 5$ ).

#### Presynaptic group II receptor inhibits mEPSCs

The addition of cadmium not only blocked the excitatory effect of ACPD but also revealed an ACPD-induced inhibition of transmitter release (Fig. 2, *A* and *B*). In 10 recordings made in the presence of cadmium, ACPD inhibited the mEPSC frequency by  $38 \pm 12\%$  but did not affect the amplitude or time course of the mEPSCs (Fig. 2*C*). These results imply that ACPD acts presynaptically by inhibiting a step in the release process that is downstream of calcium influx through voltage-gated calcium channels. The group-II-selective agonist DCG-IV (Hayashi et al. 1993), but not the group-I-selective DHPG, mimicked the inhibitory effect of ACPD (Fig. 2*D*). The mEPSC frequency inhibition due to 2  $\mu$ M DCG-IV averaged  $39 \pm 7\%$  ( $n = 10$ ). The 2  $\mu$ M concentration was apparently saturating for the DCG-IV effect, because roughly the same amount of inhibition ( $48 \pm 11\%$ ) was observed with 8  $\mu$ M DCG-IV ( $n = 4$ ). DCG-IV probably activated mGluR2 in our experiments, because mGluR2 is the only group II receptor expressed in mitral cells (Ohishi et al. 1993; Tanabe et al. 1993). The action of DCG-IV was not blocked by either MCPG (500  $\mu$ M,  $n = 6$ ) or MAP-4 (500  $\mu$ M,  $n = 5$ ), both of which can antagonize glutamate activation of mGluR2 in expression systems (Gomez et al. 1996; Hayashi et al. 1994). However, the concentrations required for these relatively weak competitive antagonists to inhibit DCG-IV on mGluRs in mitral cells are not known.

#### Presynaptic group III receptor inhibits mEPSCs

The group-III-selective agonist L-AP4 (100  $\mu$ M) also caused inhibition of transmitter release in mitral cells (Fig. 3, *A* and *B*). In 10 cells, L-AP4 inhibited the mEPSC frequency by  $40 \pm 4\%$  without affecting the amplitude or time course of mEPSCs (Fig. 3*C*). L-AP4, like ACPD and DCG-IV, apparently acts on step(s) in the release process downstream from calcium influx, because L-AP4 caused the same magnitude of inhibition whether or not the experiments were performed in cadmium (Fig. 3*D*).

Of the group III receptors, both mGluR7 and mGluR8 have been localized to the axon terminals of mitral cells (Kinoshita et al. 1996; Kinzie et al. 1997). However, in expression systems, mGluR8 is 400-fold more sensitive to L-AP4 than mGluR7. The half-maximal effective concentrations for mGluR8 and mGluR7 are 0.4 and 160  $\mu$ M, respectively (Duvoisin et al. 1995; Saugstad et al. 1994). Thus we attempted to determine whether mGluR7 or mGluR8 was responsible for the presynaptic inhibition by analyzing the concentration dependence of L-AP4. An mGluR7-mediated effect would predict greater inhibition due to a high (1 mM) concentration of L-AP4, which should activate both mGluR7

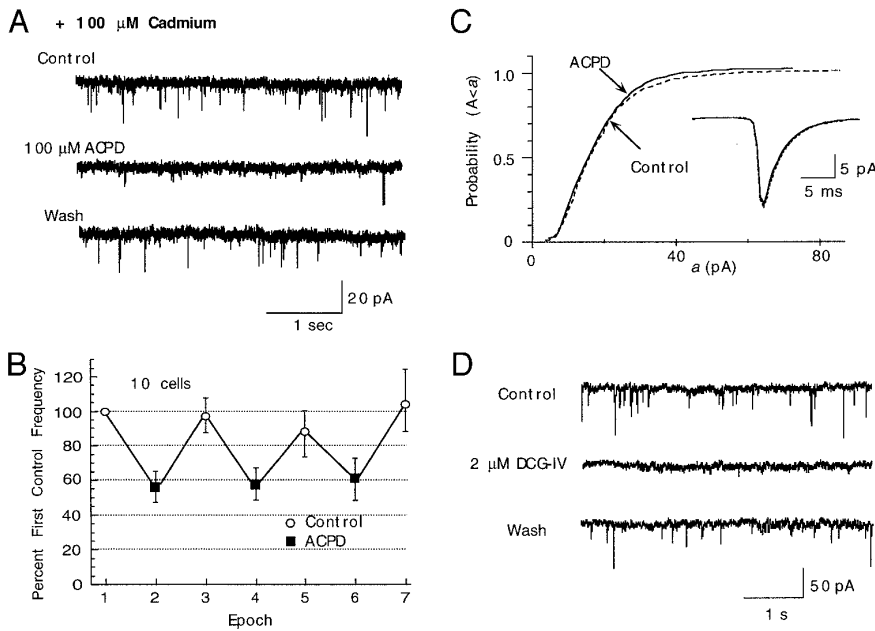


FIG. 2. Group II mGluR causes presynaptic inhibition. *A*: in the presence of  $100 \mu\text{M}$  cadmium, ACPD ( $100 \mu\text{M}$ ) reversibly reduced the mEPSC frequency in this cell. *B*: plot reflects average mEPSC frequencies in 10 cells in which ACPD was applied  $\geq 3$  times (for 30–60 s), each separated by a wash period. mEPSC frequencies were normalized to the frequency during the 1st control period. *C*: cumulative amplitude histograms constructed from the recording in *A* show that ACPD (—) caused no change in the mEPSC amplitude. Control and ACPD histograms reflect 652 and 830 mEPSCs, respectively. *Inset*: ACPD (—) also caused no change in the averaged mEPSC time course compared with control (---). *D*: the group-II-selective agonist 2-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV,  $2 \mu\text{M}$ ) also reduced the mEPSC frequency.

and mGluR8, than to a low ( $5 \mu\text{M}$ ) concentration, which should activate only mGluR8. In fact, as shown in Fig. 4*A*,  $5 \mu\text{M}$  L-AP4 caused the same magnitude of inhibition as  $1 \text{ mM}$  L-AP4. L-AP4-induced inhibition was also antagonized by MAP-4 ( $500 \mu\text{M}$ ; Fig. 4*B*), which antagonizes the action of glutamate on mGluR8 in expression systems, but not mGluR7 (Saugstad et al. 1997). Additionally, the L-AP4-induced inhibition was mimicked by L-CCG-I ( $2 \mu\text{M}$ ), an effect that was antagonized by MCPG and MAP-4 (Fig. 4*C*). An agonist action for L-CCG-I and antagonist actions for MCPG and MAP-4 have been demonstrated for mGluR8, but not mGluR7 (Gomez et al. 1996; Saugstad et al. 1997). The results with L-CCG-I, however, are not definitive, because L-CCG-I, MCPG, and MAP-4 also act on mGluR2.

The results with DCG-IV and L-AP4 imply that there are

two receptors in mitral cells—a group II receptor (probably mGluR2) and a group III receptor (probably mGluR8)—that mediate inhibition of transmitter release. To test whether the effects of these two receptors were additive, we compared the effect of ACPD, which activates mGluR2 and mGluR8, with that of L-AP4, which activates only mGluR8. For these two agonists, the inhibition of the mEPSC frequency was indistinguishable ( $51 \pm 10\%$  and  $48 \pm 13\%$ , respectively;  $n = 5$ ). In four other cells, similar magnitudes of mEPSC inhibition ( $52 \pm 11\%$  and  $46 \pm 4\%$ , respectively) were caused by L-CCG-I and DCG-IV ( $8 \mu\text{M}$ ). The fact that activation of both mGluR2 and mGluR8 caused no more inhibition than selective activation of either receptor suggests that mGluR2 and mGluR8 converge on the same step in the release process.

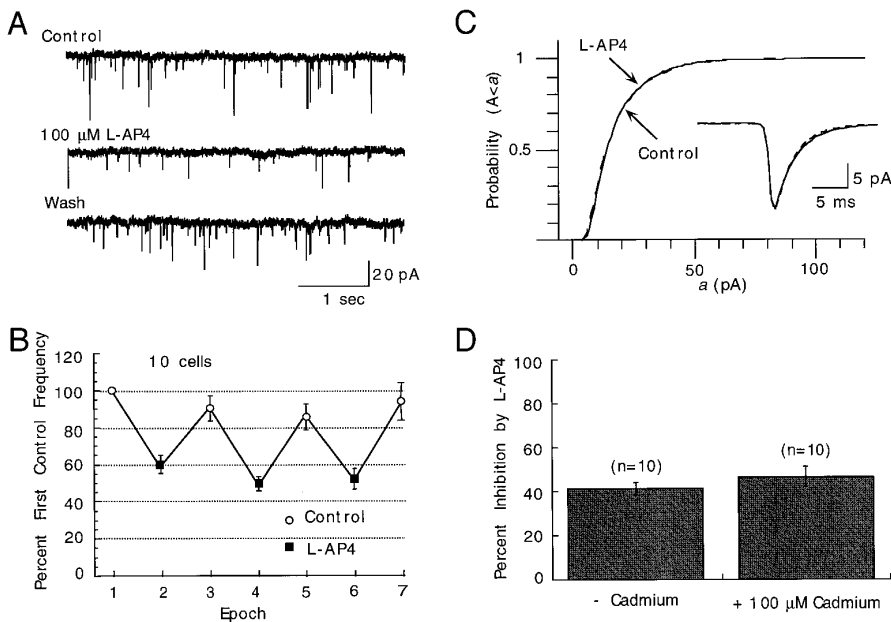


FIG. 3. Group III mGluR causes presynaptic inhibition. *A* and *B*: the group-III-specific mGluR agonist L-2-amino-4-phosphonobutyric acid (L-AP4,  $100 \mu\text{M}$ ) reduced the mEPSC frequency. The inhibition was reversible and showed no apparent desensitization, as shown in the averaged data from 10 cells. *C*: L-AP4 (—) caused no change in the mEPSC amplitude and time course compared with control conditions (---). Control and L-AP4 histograms reflect 2,210 and 1,344 mEPSCs, respectively. *D*: L-AP4 caused the same magnitude of inhibition in the absence and presence of cadmium ( $100 \mu\text{M}$ ).

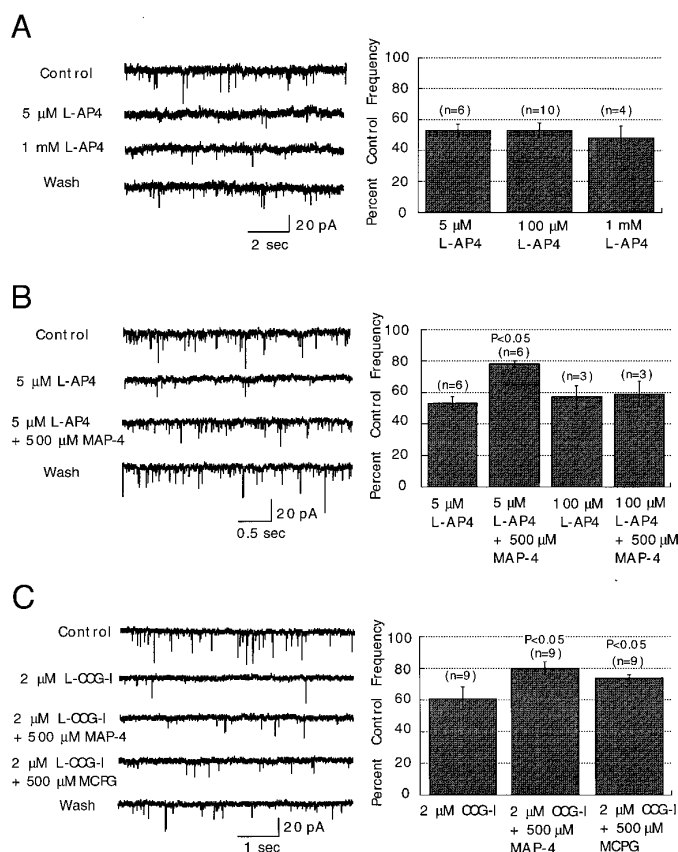


FIG. 4. Actions of L-AP4 are more consistent with activation of mGluR8. *A*: low (5  $\mu$ M) and high (1 mM) concentrations of L-AP4 reduced the mEPSC frequency by a similar amount. *B*: methyl-aminophosphonobutyric acid (MAP-4; 500  $\mu$ M) antagonized the effect of 5  $\mu$ M L-AP4 (paired *t*-test,  $n = 6$ ) but not 100  $\mu$ M L-AP4, consistent with competitive antagonism. *C*: L-CCG-I (2  $\mu$ M) also caused an inhibition of the mEPSC frequency ( $n = 9$ ), which was partially antagonized by MAP-4 (500  $\mu$ M) and MCPG (500  $\mu$ M).

#### Is L-AP4-induced presynaptic inhibition mediated by inhibition of adenylate cyclase?

In expression systems, group II and group III receptors are negatively coupled to adenylate cyclase via a pertussis toxin (PTx)-sensitive mechanism (Schoepp and Conn 1993). In mitral cells, the L-AP4-induced reduction in mEPSC frequency was also abolished by PTx. In cells that had been pretreated with PTx (250 ng/ml, 20 h), L-AP4 caused an  $8 \pm 8\%$  ( $n = 7$ ) reduction in the mEPSC frequency. This suggests that  $G_i$  or  $G_o$  mediates synaptic inhibition; thus we tested whether inhibition of adenylate cyclase was responsible for L-AP4-mediated inhibition. Increasing adenosine 3',5'-cyclic monophosphate (cAMP) by activation of adenylate cyclase with forskolin (50  $\mu$ M for as long as 10 min) or inhibiting phosphodiesterase with IBMX (50  $\mu$ M for as long as 15 min) failed to cause an increase in the mEPSC frequency (see the forskolin effect in Fig. 5A). In two other cells that had been pretreated with IBMX, forskolin also did not change the mEPSC frequency. The inability of forskolin or IBMX to increase the mEPSC frequency is, however, not inconsistent with a cAMP-mediated effect of L-AP4, if basal cAMP levels in mitral cells are sufficient to maximally enhance transmitter release. However, incuba-

tion of mitral cells with IBMX did not occlude the L-AP4-mediated inhibition of mEPSCs (Fig. 5B). Forskolin did partially inhibit the L-AP4 effect (by  $42 \pm 10\%$ ;  $n = 5$ ), but the same magnitude of inhibition of the L-AP4 effect was induced by the inactive analogue 1,9 dideoxy-forskolin. Thus our data suggest that inhibition of cAMP is not responsible for presynaptic inhibition by L-AP4.

#### Does L-AP4 inhibit transmitter release by a calcium-channel-dependent mechanism?

The inhibition of mEPSCs by L-AP4 in the presence of cadmium indicates that calcium influx is not required for presynaptic inhibition. However, in mitral cells, L-AP4 causes a reduction in whole cell calcium currents, as well as inhibition of evoked release (Trombley and Westbrook 1992), suggesting that a reduction in calcium influx might contribute to presynaptic inhibition. We tested this possibility by evaluating the magnitude of the L-AP4 inhibition while recording mEPSCs in high extracellular potassium. Similar to what has been observed in other cells (Doze et al. 1995; Scanziani et al. 1995), raising extracellular  $K^+$  from 2.5 to 20 mM caused a robust increase in the mEPSC frequency ( $220 \pm 50\%$ ;  $n = 6$ ). The high- $K^+$ -induced increase in the mEPSC frequency was blocked by cadmium, suggesting that it was caused by depolarization-induced opening of voltage-gated calcium channels. If an L-AP4-induced reduction in calcium entry affects transmitter release, we would expect the magnitude of L-AP4-induced inhibition to be greater under conditions in which calcium entry is allowed (in high  $K^+$ ) compared with that observed in low  $K^+$  (plus cadmium). However, in six cells, the amount of L-AP4-induced inhibition in high  $K^+$  ( $28 \pm 9\%$ ) was indistinguishable from inhibition in low  $K^+$  ( $36 \pm 7\%$ ), suggesting that L-AP4-induced reduction in calcium influx through calcium channels is not required to achieve the observed magnitude of mEPSC inhibition.

#### DISCUSSION

Mitral cells express members of all three groups of mGluRs: mGluR1, 2, 7, and 8. By evaluating the effect of selective mGluR agonists and antagonists on mEPSCs in cultured mitral cells, we identified modulatory functions for each receptor group on the release of transmitter from mitral cell axon terminals. Group I receptors enhanced transmitter release, whereas group II/III receptors caused presynaptic inhibition of release. Despite the well-established inhibitory effects of group II/III receptors on cAMP- and voltage-dependent calcium currents, the inhibition of mEPSC frequency by these receptors was independent of these effectors.

#### Pharmacology of mGluR actions on mitral cells

The nonselective agonist ACPD and the group-I-selective agonist DHPG caused a large increase in mEPSC frequency, implying that the activation of a group I mGluR (probably mGluR1) induces excitation of mitral cells. Group I receptors can increase neuronal excitability by several mechanisms including closure of potassium channels and opening of nonselective cation channels (Charpak et al. 1990; Crépel

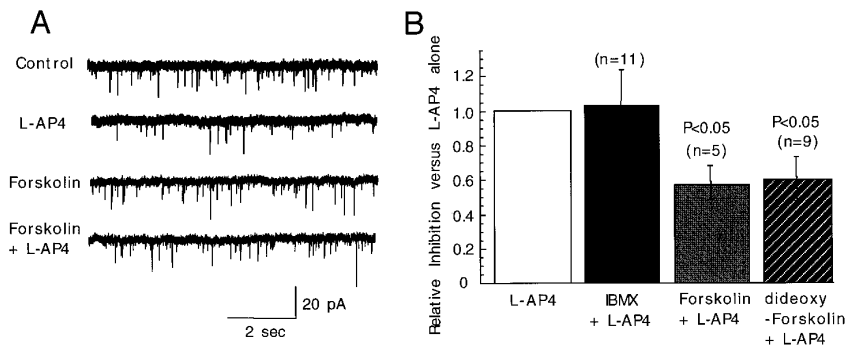


FIG. 5. L-AP4-induced presynaptic inhibition is independent of adenosine 3',5'-cyclic monophosphate (cAMP). *A*: forskolin failed to cause an increase in mEPSC frequency compared with control in 1 cell during successive applications of L-AP4 (50  $\mu$ M), forskolin (50  $\mu$ M), and L-AP4 plus forskolin (both at 50  $\mu$ M). *B*: forskolin ( $n = 5$ ) or the inactive analogue dideoxy-forskolin ( $n = 9$ ) did cause a nonspecific reduction in the effect of L-AP4, but preincubation with 3-isobutyl-1-methylxanthine (IBMX; 50  $\mu$ M) did not block the action of L-AP4. L-AP4-induced mEPSC inhibition in the presence of the indicated drugs (2nd, 3rd, and 4th bars) was normalized to the L-AP4-induced inhibition in absence of drug (scaled to 1.0 in the 1st bar).

et al. 1994). On the basis of the inhibition by intracellular cesium and the conductance decrease associated with ACPD- and DHPG-induced inward currents, closure of potassium channels was probably responsible for mGluR1-mediated excitation in mitral cells. Group I mGluRs primarily exert postsynaptic actions, although presynaptic facilitation of release has been reported (Herrero et al. 1992). In the olfactory bulb of the intact animal, mGluR1 is localized to the cell body and dendrites of mitral cells (Martin et al. 1992; van den Pol. 1995). We cannot exclude the possibility that mGluR1 is expressed in the terminals of mitral cells in culture, but the observed mGluR1-mediated increase in mEPSCs is most consistent with a postsynaptic effect on the excitability of adjacent mitral cells. This depolarization is presumably passively conducted from the somadendritic compartment to the terminals, yielding an increase in transmitter release.

For technical reasons, we were limited in our mEPSC amplitude measurements to the subset of cells that failed to respond to these drugs with a large mEPSC frequency increase and also to recordings made in the presence of cadmium. Thus ACPD and DHPG could have affected the mEPSC amplitude in the subset of cells that had an excitatory response to these drugs and also in a way that relies on calcium influx through calcium channels. However, our results suggest that mGluR1 activation has no effect on the postsynaptic responsiveness of AMPA receptors in mitral cells.

Presynaptic inhibition of transmitter release by group II and group III mGluR activation has been reported in a number of neurons (Baskys and Malenka 1991; Forsythe and Clements 1990; Gereau and Conn 1995; Hayashi et al. 1993; Lovinger and McCool 1995; Mayer and Westbrook 1987; Salt and Eaton 1995; Trombley and Westbrook 1992). In our experiments, DCG-IV and L-AP4 caused inhibition of the mEPSC frequency, implying that a group II and a group III receptor induce presynaptic inhibition of transmitter release in mitral cells. Although DCG-IV and L-AP4 appear to be group selective, we cannot completely exclude that they act through the same receptor, because some putative group-II-selective agonists such as L-CCG-I also activate some group III receptors (Saugstad et al. 1997). Agonists that activate both group II and group III receptors (ACPD and L-CCG-I) caused no more inhibition than group-selective agonists. Although this result is consistent with a single receptor, it could also occur if the group II and group III mGluRs share the same effector.

The group II receptor that mediates inhibition is likely to

be mGluR2, because it is the only group II receptor expressed in mitral cells (Ohishi et al. 1993; Tanabe et al. 1993). The axon terminals of mitral cells express two different group III receptors, mGluR7 and mGluR8 (Kinoshita et al. 1996; Kinzie et al. 1997). The low micromolar sensitivity of mEPSCs to L-AP4 implies that mGluR8 is at least partially responsible for the presynaptic inhibition, but we cannot exclude a role for mGluR7. For example, the L-AP4 sensitivities of mGluR7 and mGluR8 in neurons could be different from those in expression systems, e.g., if the efficiency of receptor/effector coupling is different. Likewise, if mGluR7 and mGluR8 are coupled to the same effector, L-AP4 could saturate mGluR8 receptors and occlude the effect of mGluR7 activation.

#### Mechanisms of presynaptic inhibition

Although several mGluR subtypes can reduce calcium influx, both DCG-IV and L-AP4 inhibited the mEPSC frequency in the presence of cadmium, implying that group II and group III receptors in mitral cells inhibit step(s) in the transmitter release process that are downstream of calcium influx through voltage-gated calcium channels. Such downstream presynaptic inhibitory effects on the release process have been reported previously for the nonselective mGluR agonist ACPD (Scanziani et al. 1995; Tyler and Lovinger 1995) and for the group-III-selective agonist L-AP4 (Gereau and Conn 1995). In other pathways, both calcium-channel-dependent and -independent mechanisms of inhibition occur. For example, in cerebellar granule cells, GABA<sub>B</sub> receptors act via calcium-channel-dependent and -independent mechanisms, but the adenosine receptor uses exclusively a calcium-channel-dependent mechanism (Dittman and Regehr 1996). In hippocampal CA1 interneurons, GABA<sub>B</sub> receptor activation reduces the miniature inhibitory postsynaptic current frequency only in high K<sup>+</sup>, implying an exclusively calcium-channel-dependent mechanism (Doze et al. 1995). In contrast, muscarinic- and mGluR-mediated inhibition of mEPSCs in hippocampal CA3 pyramidal neurons is the same in low and high K<sup>+</sup> (Scanziani et al. 1995). In mitral cells, group III receptor activation has been shown to inhibit calcium influx (Trombley and Westbrook 1992). However, in our experiments, L-AP4 caused the same magnitude of mEPSC inhibition in low and high K<sup>+</sup>. Thus the action of a group III mGluR on calcium influx appears not to be responsible for inhibition of mEPSCs.

Can our results obtained with mEPSCs be extrapolated to evoked synaptic responses? One obvious difference is that

mEPSCs do not require calcium influx. We attempted to mimic evoked release with the use of high  $K^+$ . However, calcium entry under continuous depolarizing conditions is likely to be different from that occurring during an action potential. High  $K^+$  could produce calcium concentrations in terminals that saturate the release mechanism, which, because L-AP4 produces incomplete inhibition of calcium currents (Trombley and Westbrook 1992), could mask a calcium-channel-dependent component of inhibition. However, the magnitude of mEPSCs inhibition in our experiments (averaging 47% in cadmium; Fig. 3D) closely matched the magnitude of inhibition of evoked release observed previously (47%) (Trombley and Westbrook 1992). Thus inhibition by a mechanism downstream of calcium influx appears sufficient to account for the effect of L-AP4 on evoked release.

We were unable to define the intermediate steps between activation of a G protein and inhibition of mEPSCs by group III receptor activation, although we excluded the most likely candidate. Group III receptors are negatively coupled to adenylate cyclase in expression systems, but inhibition of mEPSCs did not appear to be due to cAMP inhibition. Some previous tests of group III receptor activation on cAMP production in neurons have also yielded negative results (Prézeau et al. 1994). Additionally, effector coupling in neurons need not match that in expression systems, e.g., the group III mGluR6 couples to a guanosine 3',5'-cyclic monophosphate-specific phosphodiesterase in retinal cells (Nakanishi 1994). In some cases,  $G_i/G_o$  can act directly on effectors (Brown and Birnbaumer 1990). Thus the  $G_i/G_o$  protein coupled to a group III mGluR in mitral cell axon terminals may interact directly with one of the proteins that constitute the transmitter release machinery. Direct G-protein-mediated mechanisms such as muscarinic activation of potassium current occur within 150 ms (Yatani and Brown 1989). If the direct effect of L-AP4 activation occurs with a similar latency, the inhibition could affect release during a single burst of action potentials.

#### *Implications of mGluR activation on mitral cell function*

In cerebellar Purkinje cells, activation of dendritic mGluR1 at parallel fiber synapses induces a slow but robust membrane depolarization that is presumably integrated by the cell together with fast AMPA-receptor-mediated excitatory postsynaptic potentials (Batchelor and Garthwaite 1997). For mitral cells, mGluR1 in primary dendrites is postsynaptic to glutamate-releasing olfactory nerve terminals and could have a similar excitatory function. Within secondary dendrites, mGluR1 is presynaptic at dendrodendritic synapses with granule cells. Glutamate release at these synapses could thus have an additional local, autoexcitatory effect on transmitter release (van den Pol 1995). A glutamate-induced local or cell-wide depolarization of the mitral cell would counteract reciprocal inhibition caused by input from granule and periglomerular cells. Such excitatory effects would be specific to the activated mitral cell and thus would enhance the magnitude of lateral inhibition within the olfactory bulb.

Presynaptic inhibition caused by the activation of mGluR2 and mGluR8 would be expected to inhibit glutamate release from the axon terminals of the activated mitral cell or from

neighboring mitral cells. Why would a single mitral cell terminal have two presynaptic receptors that act on the same step in the release process? One possible explanation is that the two receptors are located in different sets of terminals. This might explain why saturating concentrations of DCG-IV and L-AP4 cause only ~50% inhibition of the mEPSC frequency. However, we found that agonists such as ACPD and L-CCG-I, which activate both mGluR2 and mGluR8, also produce only 50% inhibition. Thus either all of the terminals have the full complement of receptors and the maximal 50% inhibition is due to the limitations of the inhibitory mechanism, or alternatively, 50% of the terminals have both mGluR2 and mGluR8 and 50% have neither receptor.

If both mGluR2 and mGluR8 are located within a single axon terminal, the two receptors might play different roles based on differential localization. Both mGluR7 and mGluR8 have been localized to the active zone of mitral cell axon terminals. Although the subcellular localization of mGluR2 in mitral cells is not known, in hippocampal neurons mGluR2 is localized at the preterminal axon at some distance from the release sites (Shigemoto et al. 1996b). Thus, although mGluR8 (or 7) could be activated after a single stimulus, mGluR2 receptors at a site away from the release site could be activated only under higher release conditions (e.g., following a train of action potentials). A role for mGluR7 is a particularly compelling possibility, because mGluR7 in expression systems is much less sensitive to glutamate compared with other mGluRs (median effective concentration = 1 mM) (Okamoto et al. 1994). A receptor with a high glutamate sensitivity (e.g., mGluR2) could be activated heterosynaptically, whereas mGluR7 would be activated homosynaptically, allowing inhibition to be specific to the sites of transmitter release (Shigemoto et al. 1996a).

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