Membrane Potential and Intracellular Ca^{2+} Oscillations Activated by mGluRs in Hippocampal Stratum Oriens/Alveus Interneurons

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Woodhall, Gavin, Christine E. Gee, Richard Robitaille, and Jean-Claude Lacaille. Membrane potential and intracellular Ca^{2+} oscillations activated by mGluRs in hippocampal stratum oriens/alveus interneurons. *J. Neurophysiol.* 81: 371–382, 1999. Metabotropic glutamate receptors (mGluRs) are expressed heterogeneously in hippocampal interneurons, and their signal transduction cascades remain largely unclear. We characterized an oscillatory response activated by the mGluR agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) in hippocampal interneurons of stratum oriens-alveus (OA) with simultaneous whole cell current-clamp recordings and intracellular Ca^{2+} imaging with confocal microscopy. 1S,3R-ACPD induced oscillatory membrane depolarizations and rises in intracellular Ca^{2+} that persisted in tetrodotoxin and were blocked by the antagonist of group I and II mGluRs (S)-α-methyl-4-carboxyphenylglycine. Membrane depolarizations and intracellular Ca^{2+} rises were blocked by extracellular Cd^{2+} and in Ca^{2+}-free medium. mGluR responses therefore required Ca^{2+} influx via voltage-gated Ca^{2+} channels. 1S,3R-ACPD responses were also antagonized by depleting intracellular stores with thapsigargin and ryanodine, indicating that Ca^{2+} release from intracellular stores was also necessary. These data suggest that oscillatory responses generated by group I/II mGluRs involve a coupling of Ca^{2+} entry through voltage-gated Ca^{2+} channels and Ca^{2+} release from internal stores. In contrast, 1S,3R-ACPD evoked only smaller depolarizations and intracellular Ca^{2+} rises, with no oscillations, in other hippocampal interneurons located in or near stratum lacunosum-moleculare. Thus mGluR-mediated oscillatory responses are specifically expressed in certain interneuron subtypes. This heterogeneous expression of glutamate and Ca^{2+} signaling pathways in specific interneurons may be relevant to their selective vulnerability to excitotoxicity.

INTRODUCTION

A local network of GABAergic interneurons controls the excitability of pyramidal cells in the hippocampus (Buhl et al. 1994; Buzsáki and Chrobak 1995; Lacaille et al. 1989). Much is known about the morphology of these interneurons (Buhl et al. 1994; Lorento de No 1934; Ramon y Cajal 1911), their intrinsic properties (Lacaille and Schwartzkroin 1988a; Maccaferri and McBain 1996; Schwartzkroin and Mathers 1978), and the specific domain of pyramidal cells preferentially targeted by their synapses (Buhl et al. 1994; Freund and Buzsáki 1996; McBain et al. 1994). However, the excitatory postsynaptic mechanisms that control the activity of inhibitory interneurons remain largely unclear (McBain and Dingledine 1993; Morin et al. 1996; Sah et al. 1990). Interneurons of the hippocampal CA1 region receive major excitatory inputs from CA3 and/or CA1 pyramidal cells (Blasco-Ibanez and Freund 1995; Lacaille and Schwartzkroin 1988; Lacaille et al. 1987; Maccaferri and McBain 1995). These synapses bear *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors subserving fast synaptic transmission (Perouansky and Yaari 1993; Sah et al. 1990). Interestingly, mGluRs are expressed heterogeneously in interneurons and have cell-specific pre- and postsynaptic modulatory roles (McBain et al. 1994). Hence it is important to determine the signaling cascades that underlie these cell-specific actions.

Activation of mGluRs leads, via their coupling through G-proteins (Nicoletti et al. 1986), to complex second messenger cascades, which, depending on the mGluR subtype activated, may involve production of inositol triphosphate (IP_3) and diacylglycerol (DAG) (Houamed et al. 1991; Masu et al. 1991), negative modulation of adenylyl-cyclase activity (Tanabe et al. 1992), and Ca^{2+} release from internal stores (Murphy and Miller 1988). In the hippocampal CA1 region, mRNA expression for the type 1 mGluR (mGluR1) and immunoreactivity for the isoform mGluR1α are specifically found in interneurons of stratum oriens-alveus (OA) (Baude et al. 1993; Fotuhi et al. 1994; Masu et al. 1991). Also, mGluR1s were identified at perisynaptic locations on the dendrites of inhibitory interneurons in OA and may thus be coupled to IP_3 production and release of intracellular Ca^{2+} in these cells (Baude et al. 1993; Luján et al. 1996). However, because IP_3 receptors appear absent from interneurons in these layers, mGluR1 effects may alternatively involve phospholipase C-mediated production of DAG and subsequent activation of protein kinase C (Fotuhi et al. 1993).

Activation of mGluRs by the agonist 1S,3R-ACPD produces oscillatory inward currents in hippocampal horizontal interneurons in OA (McBain et al. 1994). Microapplications of glutamate were shown to elicit oscillatory rises in intracellular Ca^{2+} involving mGluRs in unidentified interneurons in OA (Carmant et al. 1997); however, the link between mGluR activation and intracellular Ca^{2+} levels remains unclear in hippocampal interneurons. Thus the aim of these experiments was to examine, with simultaneous whole cell current-clamp recording and intracellular Ca^{2+} imaging, the actions of the class I and II mGluR agonist 1S,3R-ACPD on hippocampal interneurons in OA and determine the cellular

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events involved in these actions. Our results show cell-specific mGluR responses involving a coupling between voltage-dependent Ca^{2+} currents and Ca^{2+} release from intracellular stores.

METHODS

Slice preparation

Hippocampal slices were prepared as previously described (Carmant et al. 1997; Morin et al. 1996; Ouardouz and Lacaille 1997). Briefly, young (14–21 days) male Sprague-Dawley rats were decapitated, and the brain was removed and placed in artificial cerebrospinal fluid (aCSF) containing (in mM) 128 NaCl, 5 KCl, 2 $CaCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $MgSO_4$, and 10 glucose, pH 7.4 at 4°C for 1–3 min. Osmolarity of aCSF was adjusted to 305 mOsm with sucrose. Blocks of brain containing the hippocampus were prepared by removing the cerebellum, separating the hemispheres along the midline, partially removing the frontal cortex, and trimming the temporal cortex to form a flat surface. These blocks of brain were glued to a stage with cyanoacrylate and transverse hippocampal slices cut at 300-μm thickness with a vibratome (Cambridge Instruments). After ≥60 min of recovery in aCSF at room temperature (22°C), individual slices were transferred to a recording chamber attached to the stage of an upright laser scanning confocal microscope (Olympus BH5 and BioRad MRC-600). The chamber was perfused with aCSF at a rate of 2–3 ml/min at room temperature. In most experiments, tetrodotoxin (TTX 0.5 μM) was added to the perfusate to block voltage-dependent Na^+ channels.

Electrophysiology

Interneurons located in stratum OA or near the border of stratum radiatum and stratum lacunosum-moleculare (LM) were identified with differential interference contrast optics and electrophysiological recordings obtained with the patch-clamp technique in the whole cell configuration (Hamill et al. 1981). Patch pipettes (4–8 MΩ) were pulled from borosilicate glass (1 mm OD, A-M Systems) and filled with the following internal solution (in mM): 120 K-gluconate, 20 KCl, 4 Na-ATP, 0.4 Na-GTP, and 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, which was titrated to pH 7.2–7.25 with KOH. Osmolarity was adjusted to 275 mosm with distilled water. The pipette solution also contained 25 or 50 μM of the calcium fluorophore calcium-green-1 (hexapotassium salt, Molecular Probes) for visualization of Ca^{2+} responses with the laser scanning confocal microscope. In some cases biocytin 0.15% was added to the internal patch solution for later morphological characterization of the cell being recorded. Membrane voltage was recorded in current clamp with an Axoclamp-2B amplifier (Axon Instruments) in bridge mode, filtered at 10 kHz, digitized at 22 kHz, and recorded on videotape. Recorded signals were fed into an A/D converter (TL-1, Axon Instruments), stored on a PC, and analyzed off-line with pClamp software (Axon Instruments). The patch pipette was advanced and positioned onto a target cell under visual control with a ×40 long-range water-immersion objective (Olympus, numerical aperture 0.75) until a small indentation of the membrane could be seen. Tight seals (1–10 GΩ) were formed by brief application of negative pressure to the pipette, and the process of seal formation was monitored in voltage-clamp mode. After 1–3 min, the membrane was ruptured with negative pressure to allow whole cell access. The amplifier was immediately switched from voltage-clamp to bridge mode to obtain a measure of resting membrane potential before significant exchange of intracellular content and electrode solution could take place. No correction was made for whole cell capacitance, and junction potentials were corrected after placing the pipette in the bath. Bridge balance was adjusted with the bridge circuit and was monitored at regular intervals. Cells with a resting membrane potential more negative than −50 mV and overshooting action potentials were accepted.

Calcium imaging

Cells were dialysed with electrode solution for ≥20 min, with negative current injection (10–50 pA) to facilitate cell filling with calcium green-1. The calcium-green-1 fluorophore was excited with a 488-nm argon laser line attenuated to 1% of maximum power. Emission was detected through a low-pass emission filter with cutoff at 515 nm and was recorded with the MPL software (BioRad) of the confocal microscope. Confocal aperture was maintained at near maximum to obtain an adequate signal-to-noise ratio. Time-lapse images were recorded at a rate of 0.133 Hz to avoid possible phototoxic damage to the dye-filled cell. Images were stored on optical disk and analyzed off-line with Cfocal software (kindly provided by Dr. M. P. Charlton, University of Toronto). Pseudocolor confocal images were coded so that blue indicates low level and red indicates high level of Ca^{2+}. For each image, fluorescence intensity (F) was averaged over a delimited area of the soma. Changes in fluorescence (ΔF) were measured as changes in baseline fluorescence and expressed as

$$\%\Delta F/F = [(F_{post} - F_{rest})/F_{rest}] \times 100$$

To facilitate comparison among complex oscillatory waveforms, the total area under the Ca^{2+} response was measured with a trapezoidal rule-based algorithm (Sigma Plot, Jandel Scientific). Timing of electrophysiological and imaging data were synchronized with pClamp software. A current injection protocol was used to depolarize membrane potential from −65 to −10 mV. This protocol elicited a voltage-dependent Ca^{2+} signal that was used to assess cell viability. Healthy cells responded with a 40% $\Delta F/F$, and any cell that exhibited abnormally low fluorescence response was rejected.

Histology

After recording, slices containing biocytin-filled cells were transferred to a freshly prepared solution of 4% paraformaldehyde and fixed overnight at 4°C. After washing in 0.1 M phosphate buffer (PB) slices were embedded in agarose and cut in 30- to 50-μm thick sections on a vibratome (Oxford Series 1000, Scott Scientific). Sections were treated with 1% H_2O_2 for 20 min to eliminate endogenous peroxidases. Individual sections were then rinsed for 5 min in four changes of PB saline containing 0.1% Triton and incubated for 24 h with avidin–biotin complex (1:200, ABC kit, Vectastain). After rinsing for 1 h in Tris-buffered saline (pH 7.6), sections were incubated for 12 min in a Tris-buffered solution containing 0.05% diaminobenzidine (DAB), 7.5 mM $NiSO_4$, 0.0025% H_2O_2, and 0.02% imidazoline (pH 7.6). Sections were rinsed in Tris-buffered saline at pH 7.6, placed on a microscope slide, and air dried for 24 h before mounting in DPX. Biocytin-filled neurons were drawn with a camera lucida.

Pharmacology and statistical analysis

1S,3R-1-aminocyclopentane-1,3dicarboxylic acid (1S,3R-ACPD) and (S)-α-methyl-4-carboxyphenylglycine [(S)-MCPG] were obtained from Tocris-Cookson. Ryanodine and thapsigargin were obtained from RBI. TTX and other chemicals were obtained from Sigma (St. Louis, MO). Numerical data in the text and figures are expressed as means ± SE. Student's *t*-tests and analysis of variance were used to compare treatments between 2 or more groups, respectively.

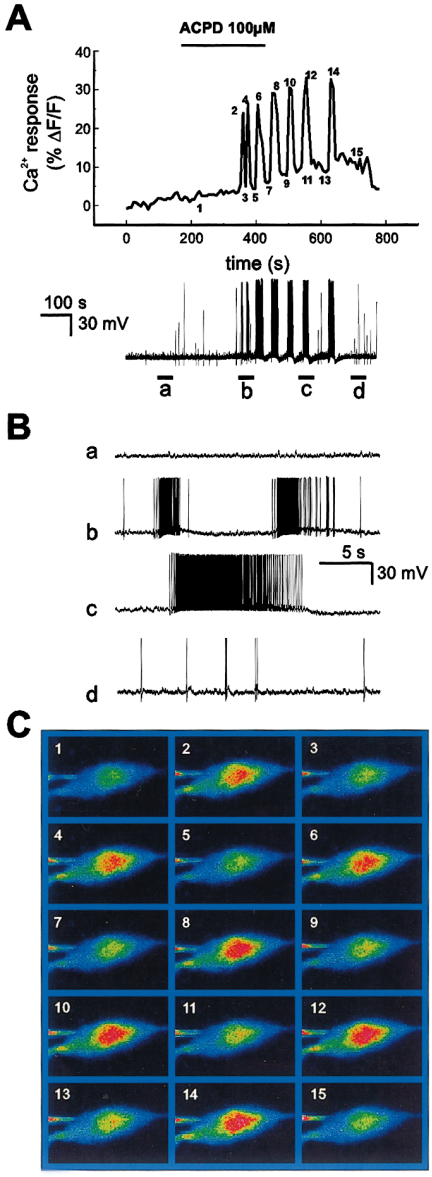

Fig. 1

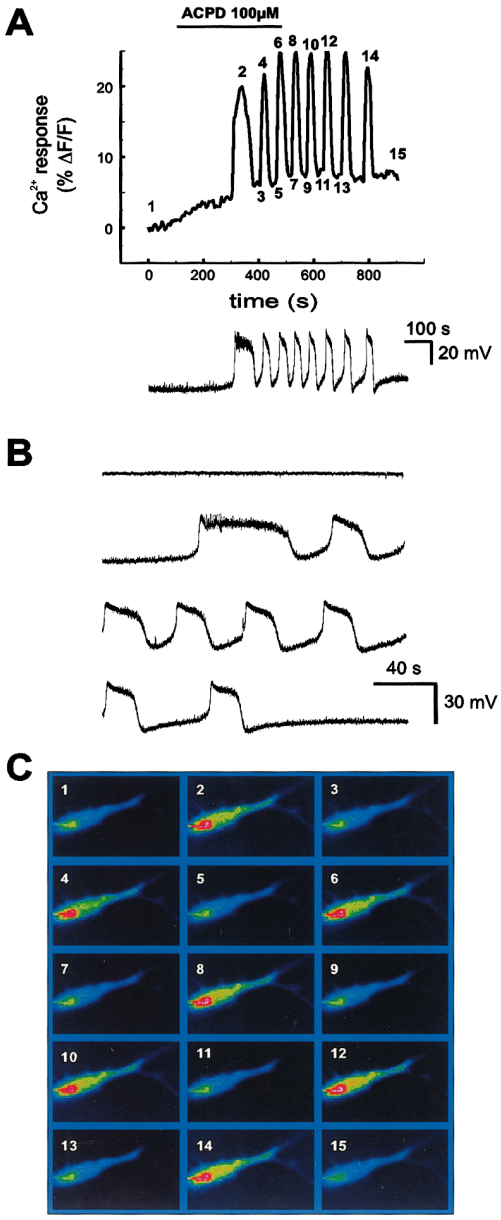

Fig. 2

FIG. 1. Oscillatory responses elicited in oriens-alveus (OA) cells by 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD). *A*: bath application of 1S,3R-ACPD (at the time indicated by the bar) depolarized the cell, induced burst firing (*bottom trace*), and increased intracellular Ca^{2+} levels (*top graph*). Responses to 1S,3R-ACPD were characterized by multiple, repetitive episodes of burst firing and elevated intracellular Ca^{2+}, which continued after washout of the drug. Membrane potential and Ca^{2+} response were recorded simultaneously and are shown on the same timescale. *B*: selected portions of the current-clamp record (indicated by *a*–*d* in *A*) are shown on an expanded time scale. *C*: pseudocolor images (blue–red scale) of intracellular Ca^{2+} levels in an OA cell, corresponding to time points indicated by the numbers 1–15 in *A*. After 1S,3R-ACPD, intracellular Ca^{2+} levels increased repetitively in the soma and proximal dendrites of the cell.

FIG. 2. Oscillatory plateau potentials and intracellular Ca^{2+} rises evoked by 1S,3R-ACPD in tetrodotoxin. *A*: in the presence of 0.5 μM tetrodotoxin (TTX), application of 1S,3R-ACPD (at time indicated by bar) elicited large amplitude plateau-like depolarizations (*bottom trace*) that were of variable duration and accompanied by rises in intracellular Ca^{2+} levels (*top graph*). *B*: membrane potential record of *A* is shown on an expanded time scale. *C*: pseudocolor images showing the repetitive increases in intracellular Ca^{2+} levels induced by 1S,3R-ACPD in the soma and proximal dendrite of an OA cell, corresponding to time points indicated by the numbers 1–15 in *A*.

RESULTS

1S,3R-ACPD evoked oscillatory responses in OA interneurons

Under control conditions, with slices perfused with standard aCSF and cells maintained just below spike threshold with steady current injection, bath perfusion of 100 μM 1S,3R-ACPD for 3–5 min evoked oscillatory responses in eight of eight interneurons in OA (Fig. 1). Typically, an increase in membrane potential noise was observed shortly after drug application. This was followed by a membrane depolarization (10.9 ± 5.4 mV, $n = 8$), which reached threshold and triggered repetitive firing of action potentials. Intracellular Ca^{2+} levels in the soma and proximal dendrites increased in phase with the membrane depolarization and firing (Fig. 1, *A* and *C*). The membrane depolarization and associated Ca^{2+} responses were transient, occurred repetitively during and after application of 1S,3R-ACPD, and were of variable duration. The relationship among the membrane depolarization, action potential firing, and intracellular Ca^{2+} levels could not be determined more precisely because of the slow sampling rate of images (0.13 Hz) compared with that of electrophysiological recordings (22 kHz). The increase in intracellular Ca^{2+} was calculated as the area under the curve by integrating the change in fluorescence over time (7,802 ± 1, 256% $\Delta F/F \times$ s). This analysis obviated problems associated with measuring single peaks within a complex signal and facilitated comparisons among responses, which were variable with respect to peak amplitude and oscillation period. Intervals between repeated burst responses and elevations in intracellular Ca^{2+} ranged from 5 to 100 s and were associated with a return of intracellular

Ca^{2+} toward baseline levels. The oscillatory responses persisted for several minutes after washout of 1S,3R-ACPD, albeit with a decrease in frequency. In some cells (e.g., Fig. 1), the intracellular Ca^{2+} level did not return completely to baseline levels after washout of 1S,3R-ACPD. It is unlikely that the incomplete return to baseline was due to ongoing dye-loading of the cell because it was not observed often during similar 1S,3R-ACPD applications in the presence of pharmacological treatments that blocked ACPD responses (e.g., Figs. 5 and 6; see DISCUSSION). In contrast, membrane potential returned to control levels usually ≤5 min after washout of 1S,3R-ACPD. Repeated application of 1S,3R-ACPD resulted in similar depolarizing and Ca^{2+} responses (data not shown), although in some experiments the second response was slightly reduced in amplitude.

1S,3R-ACPD evoked synchronous plateau potentials and intracellular Ca^{2+} rises in TTX

Application of 1S,3R-ACPD, in the presence of 0.5 μM TTX to block voltage-dependent sodium channels, also produced oscillatory responses (Fig. 2). However, in the presence of TTX, 1S,3R-ACPD induced repetitive depolarizing plateau potentials, and the membrane depolarizations (25.5 ± 2.0 mV, $n = 27$) were significantly larger than in control aCSF (Fig. 3). These membrane depolarizations were also of variable duration (e.g., examples in Figs. 2, 4, 5, 7, and 8). The synchronous repetitive elevations in intracellular Ca^{2+} levels (Fig. 2) were not significantly different from those observed in control aCSF (Fig. 3). These data indicate that the ACPD-induced oscillatory responses were not synaptically driven events and that both the membrane potential depolarization and the increase in intracellular Ca^{2+} did not require sodium channel activation in interneurons. The larger membrane potential depolarizations evoked by 1S,3R-ACPD in the presence of TTX seem likely to be due to a decreased activation of K^+ conductances in the absence of action potentials, resulting in less shunting of depolarizations. Consistent with this possibility, McBain et al. (1994) reported similarly large depolarizations (20–30 mV) evoked by 1S,3R-ACPD in OA interneurons during whole cell recordings with internal patch solution containing Cs^+ to block potassium currents. Almost all OA cells (27/29) tested in the presence of TTX responded to 1S,3R-ACPD with significant membrane depolarizations and increases in intracellular Ca^{2+} (11,703 ± 2,108% $\Delta F/F \times$ s). There was no statistically significant difference in the magnitude, or the profile, of Ca^{2+} responses between cells in control and those in TTX-containing aCSF (Fig. 3). These similar intracellular Ca^{2+} responses, in the presence of membrane depolarizations of different amplitude, suggest that the ACPD-induced Ca^{2+} rises were not simply a consequence of membrane depolarization. The increase in membrane potential noise during ACPD application was also observed in experiments with TTX and was prominent on the slow depolarizing plateau potentials (e.g., Fig. 2*B*), suggesting a postsynaptic mechanism. Finally, in another group of cells tested in TTX, hyperpolarizing the membrane potential back to pre-ACPD levels during ACPD-induced depolarizations did not prevent the intracellular Ca^{2+} rises and did not stop the depolarizing oscillatory responses ($n = 7$ cells; data not shown). These results indicate that the intracellular Ca^{2+} rise induced by 1S,3R-ACPD was not caused merely by membrane depolarization that activated voltage-dependent Ca^{2+} channels and are consistent with the previous report of ACPD-induced oscillatory inward currents observed in OA interneurons voltage-clamped near resting membrane potential (McBain et al. 1994).

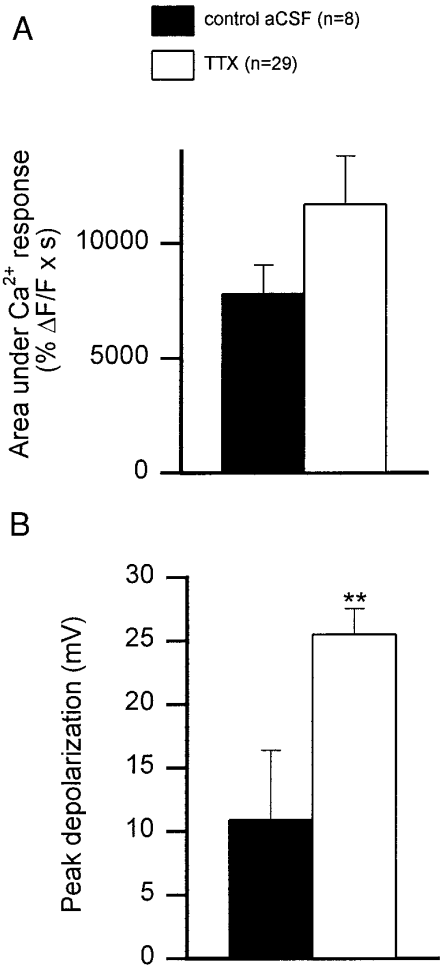

FIG. 3. Comparison of 1S,3R-ACPD responses in control and TTX-containing artificial cerebrospinal fluid (aCSF). Ca^{2+} elevations (*A*; mean area under curve) were not significantly different between OA cells in control and TTX-containing aCSF, but membrane depolarizations (*B*; peak amplitude) were significantly larger in TTX. ** $P < 0.05$, Student's *t*-test.

Block of oscillatory responses by the mGluR antagonist (S)-MCPG

To confirm that 1S,3R-ACPD responses in OA interneurons were mediated by mGluRs, the effects of the competitive antagonist of group I and II receptors (S)-MCPG were examined. 1S,3R-ACPD responses were evoked 15 min after obtaining whole cell access and again in the presence of 400 μM (S)-MCPG. All cells ($n = 7$) responded to the initial 1S,3R-ACPD application with a large membrane depolarization (31.6 ± 2.6 mV) and increase in intracellular Ca^{2+} (7,600 ± 1,445% $\Delta F/F \times$ s; Fig. 4). During superfusion with (S)-MCPG, 1S,3R-ACPD responses were significantly reduced (depolarization 3.6 ± 1.0 mV, Ca^{2+} response

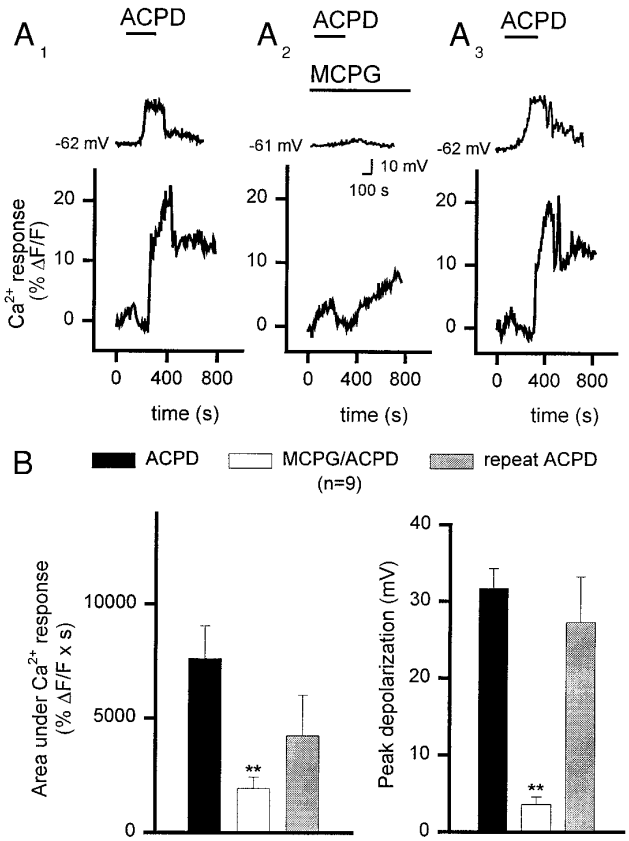

FIG. 4. Reversible block of 1S,3R-ACPD responses by the metabotropic glutamate receptor (mGluR) antagonist (S)-MCPG in OA cells. A_1: application of 1S,3R-ACPD (at time indicated by bar) evoked a membrane depolarization (*top trace*) and intracellular Ca^{2+} rise (*bottom graph*) in control aCSF. A_2: in the presence of 500 μM (S)-MCPG, the membrane depolarization and intracellular Ca^{2+} rise, evoked by a similar application 1S,3R-ACPD, were reduced in the same cell. A_3: after washout of (S)-MCPG, reapplication of 1S,3R-ACPD elicited similar membrane depolarizations and intracellular Ca^{2+} rises as in control aCSF. *B*: summary histograms for all OA cells tested ($n = 9$), showing the significant and reversible block by (S)-MCPG of membrane depolarizations (*right graph*, mean amplitude; ** $P < 0.05$, Student's *t*-test) and intracellular Ca^{2+} responses (*left graph*, mean area under the curve) evoked by 1S,3R-ACPD.

1,927 ± 520% $\Delta F/F \times$ s; $P < 0.05$) and without oscillations. After a long period of washout of (S)-MCPG (~45 min), reapplication of 1S,3R-ACPD elicited, in five of these seven cells, large membrane depolarizations (27.2 ± 5.9 mV) and Ca^{2+} responses (4,248 ± 1,762% $\Delta F/F \times$ s) that did not differ significantly from control responses (Fig. 4). These data indicate that group I or II mGluRs are involved in the depolarizing and intracellular Ca^{2+} responses evoked by 1S,3R-ACPD in OA interneurons.

1S,3R-ACPD responses require Ca^{2+} entry via voltage-dependent Ca^{2+} channels

Activation of mGluRs is known to release Ca^{2+} from intracellular stores (Murphy and Miller 1988). However, recently, activation of mGluR1s was found to affect voltage-dependent Ca^{2+} channels in cultured cerebellar neurons (Chavis et al. 1996). To assess the importance of Ca^{2+} entry via voltage-gated Ca^{2+} channels in 1S,3R-ACPD responses, experiments were carried out in aCSF with either external Ca^{2+} replaced with Mg^{2+} (nominally Ca^{2+}-free medium) or 50 μM Cd^{2+} to block voltage-gated Ca^{2+} channels. In the first series of experiments, 1S,3R-ACPD was applied first during perfusion with Ca^{2+}-free aCSF containing TTX and again after return to aCSF containing normal 2 mM Ca^{2+}. In nine of nine cells, 1S,3R-ACPD responses were significantly smaller in Ca^{2+}-free aCSF (depolarization 0.1 ± 0.1 mV, Ca^{2+} rise 1,120 ± 588% $\Delta F/F$) relative to responses after a return in normal aCSF (depolarization 19.1 ± 4.6 mV, Ca^{2+} rise 8,132 ± 3,191% $\Delta F/F \times$ s; Fig. 5). These results indicate that Ca^{2+} entry was necessary for the 1S,3R-ACPD responses. To verify that voltage-dependent Ca^{2+} channels were involved in these responses, Cd^{2+} was used in aCSF with normal Ca^{2+} concentration and without TTX. In this second series of experiments, 1S,3R-ACPD was applied first in normal aCSF and then in aCSF containing 50 μM Cd^{2+} (Fig. 6). In four cells tested, the initial 1S,3R-ACPD response in control aCSF was similar to other control experi-

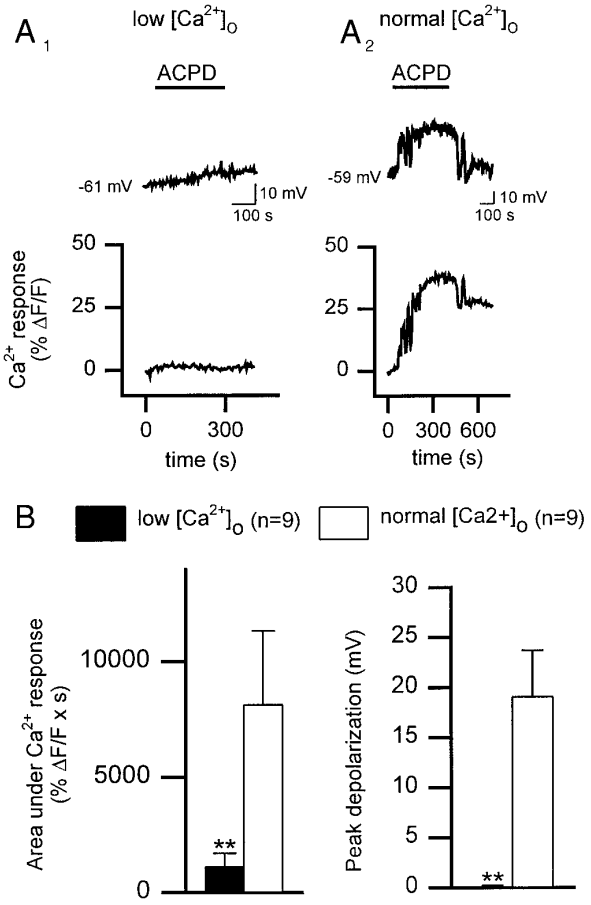

FIG. 5. Reversible block of 1S,3R-ACPD responses in Ca^{2+}-free medium in OA cells. A_1: application of 1S,3R-ACPD (at time indicated by bar) in aCSF containing 0 mM Ca^{2+} and TTX evoked very little membrane depolarization (*top trace*) and no intracellular Ca^{2+} rise (*bottom graph*). A_2: in the same cell, reapplication of 1S,3R-ACPD after the return to aCSF containing normal Ca^{2+} (2 mM) evoked large oscillatory membrane depolarizations and intracellular Ca^{2+} rises. *B*: summary histograms for all cells tested ($n = 9$) showing the significant, reversible block of the 1S,3R-ACPD responses in Ca^{2+}-free aCSF. Both the membrane depolarization (peak depolarization, *right graph*) and the Ca^{2+} response (mean area under curve, *left graph*) were significantly reduced in Ca^{2+}-free aCSF (low$[Ca^{2+}]_o$, filled bar) compared with values obtained in aCSF with normal Ca^{2+} (normal $[Ca^{2+}]_o$, open bar). ** $P < 0.05$ (Student's *t*-test).

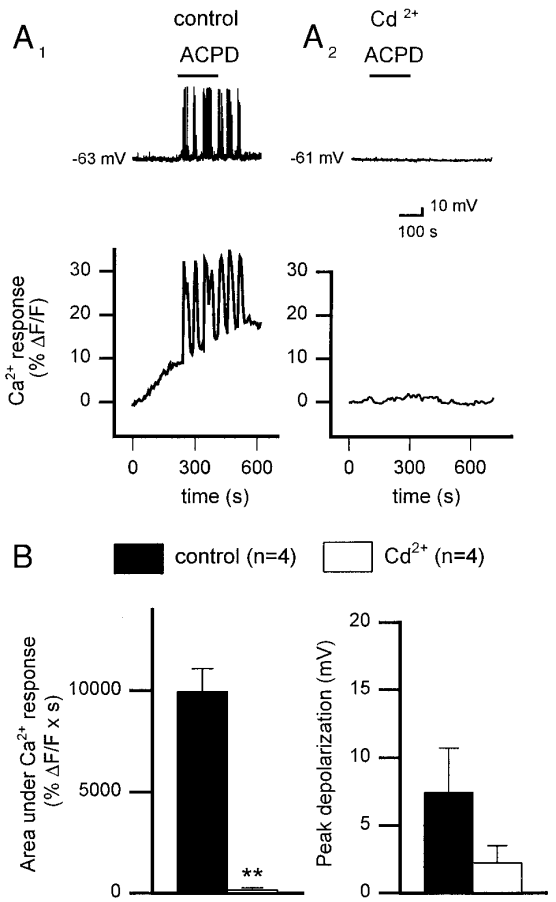

FIG. 6. Reduction of 1S,3R-ACPD responses by Cd^{2+} in OA cells. A_1: in control aCSF (without TTX), application of 1S,3R-ACPD (at time indicated by bar) evoked repetitive episodes of burst firing (*top trace*) and elevation in intracellular Ca^{2+} (*bottom graph*). A_2: membrane depolarization and the intracellular Ca^{2+} response were completely blocked in the same cell when 1S,3R-ACPD was reapplied in the presence of 50 μM Cd^{2+}. *B*: summary histograms for all cells tested ($n = 4$) showing the significant block of the Ca^{2+} response (area under the Ca^{2+} curve, *left graph*; ** $P < 0.05$ Student's *t*-test) and the reduction of the membrane depolarization (*right graph*) in the presence of Cd^{2+} (open bar) compared with control aCSF (filled bar).

ments (peak depolarization 7.4 ± 3.3 mV, Ca^{2+} rise 9,942 ± 161% $\Delta F/F \times$ s). The second 1S,3R-ACPD application in Cd^{2+} evoked a smaller depolarization (2.2 ± 1.3 mV) and Ca^{2+} rise (160 ± 106% $\Delta F/F \times$ s). The reduction of the Ca^{2+} response was significant ($P < 0.05$; Fig. 6). The reduction in ACPD-induced depolarization did not reach significance. However, in the absence of TTX, the underlying depolarization is largely underestimated (Fig. 3). Furthermore, ACPD-induced depolarizations in Cd^{2+} remained subthreshold for action potentials, indicating a marked response reduction (Fig. 6). Together these data suggest that the oscillatory 1S,3R-ACPD responses require Ca^{2+} entry through voltage-gated Ca^{2+} channels.

1S,3R-ACPD oscillatory responses require release of Ca^{2+} from intracellular stores

To examine if 1S,3R-ACPD responses also involved the release of Ca^{2+} from intracellular stores (Berridge 1991; Henzi and MacDermott 1992; Murphy and Miller 1988), ryanodine and thapsigargin were used to deplete Ca^{2+} from intracellular stores before 1S,3R-ACPD application. Ryanodine (10 μM) affects intracellular stores linked to ryanodine receptors (RyR) (Smith et al. 1988) but not IP_3-sensitive stores, whereas thapsigargin (1 μM) blocks the endoplasmic Ca^{2+}-ATPase (Sagara et al. 1992) and thus depletes both IP_3- and RyR-sensitive stores. We used both compounds because an inverse relation was reported between inositol triphosphate receptor (IP_3-R) activity and mGluR1 mRNA in cells of stratum oriens of hippocampus (Fotuhi et al. 1993), which suggests that IP_3-R mediated Ca^{2+} release from internal stores may not be significant in OA interneurons. For the first series of experiments, 1 μM thapsigargin was bath applied in the presence of TTX for 15 min, followed by the application of 1S,3R-ACPD. During the washon of thapsigargin, numerous small depolarizing events were visible in the voltage records for most cells, and a small hyperpolarization (typically 2–5 mV) often occurred. A significant increase in the baseline fluorescence signal was also observed in all cells (data not shown). Also, cells were briefly depolarized from −65 to −10 mV to ensure that a voltage-gated Ca^{2+} signal could be obtained. These observations are consistent with an accumulation of intracellular Ca^{2+} caused by depletion of internal stores, which may result in small depolarizing events caused by Ca^{2+} current activation by intracellularly released Ca^{2+} (Hoth and Penner 1992; Luckhoff and Clapham 1994; Reyes and Stanton 1996) as well as in activation of Ca^{2+}-dependent potassium currents. In seven of eight cells, thapsigargin completely blocked the 1S,3R-ACPD–induced intracellular rise in Ca^{2+} (e.g., Fig. 7; 359 ± 359% $\Delta F/F \times$ s, $n = 8$ cells). The 1S,3R-ACPD–evoked membrane depolarization was also significantly reduced, but a small depolarizing response was seen in five of these cells (2.2 ± 1.0 mV, $n = 8$ cells). In four of eight cells, reapplication of 1S,3R-ACPD in normal aCSF, 30 min after washout of thapsigargin, resulted in a small but significant intracellular Ca^{2+} rise (1,144 ± 625% $\Delta F/F \times$ s) and membrane depolarization (6.93 ± 2.8 mV), which were always oscillatory. One cell showed a complete recovery of 1S,3R-ACPD responses after thapsigargin washout for 40 min (Fig. 7A_2). These data indicate that release of Ca^{2+} from intracellular stores was necessary for the oscillatory membrane depolarization and Ca^{2+} response in OA interneurons and that voltage-gated Ca^{2+} influx alone was not sufficient to generate these responses.

To discriminate between IP_3 and ryanodine-sensitive intracellular Ca^{2+}stores in 1S,3R-ACPD responses, 10 μM ryanodine was bath applied before 1S,3R-ACPD application (in the presence of TTX). A 15-min preapplication was not effective, but 30-min preapplication of ryanodine significantly antagonized 1S,3R-ACPD responses. In 11 of 11 cells, 15-min preexposure to 10 μM ryanodine did not prevent 1S,3R-ACPD–evoked membrane depolarizations and rises in intracellular Ca^{2+}. Ca^{2+} elevations (9,741 ± 2,027% $\Delta F/F \times$ s) did not differ significantly from the control values obtained in the presence of TTX. In eight of nine cells, a second 1S,3R-ACPD response was obtained 20 min after returning to normal aCSF, and it did not differ significantly from the first Ca^{2+} response (6,770 ± 1,797% $\Delta F/F \times$ s). Membrane depolarizations coupled to these Ca^{2+} responses were not significantly different from control

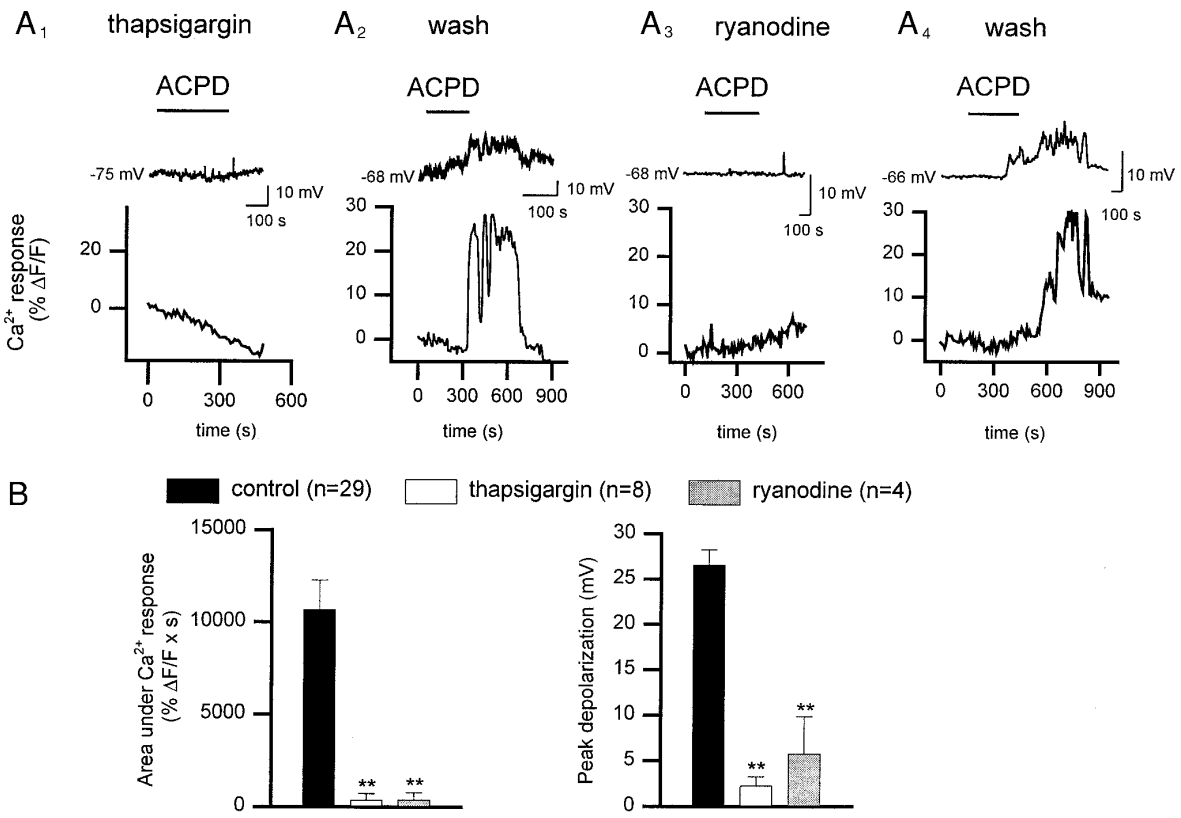

FIG. 7. Reversible block of 1S,3R-ACPD responses by depletion of intracellular Ca^{2+} stores with thapsigargin or ryanodine. A_1: application of 1S,3R-ACPD, at time indicated by bars, after pretreatment with 1 μM thapsigargin for 15 min did not elicit membrane depolarizations (*top trace*, −75 mV) or intracellular Ca^{2+} elevations (*bottom graph*). A_2: reapplication of 1S,3R-ACPD after 45 min of washout of thapsigargin evoked oscillatory membrane depolarizations and intracellular Ca^{2+} rises in the same OA cell. In this particular cell, a rundown of baseline Ca^{2+} levels occurred, which was marked during the first application of ACPD (A_1) but was also present to a lesser degree during the second application after washout of thapsigargin (A_2). The presence of this rundown during and after thapsigargin may be due to bleaching of the dye. A_3: in a different OA interneuron, 1S,3R-ACPD did not evoke changes in membrane potential (*top trace*, −68 mV) and intracellular Ca^{2+} levels (*bottom graph*) after pretreatment with 10 μM ryanodine for 30 min. A_4: reapplication of 1S,3R-ACPD elicited oscillatory membrane depolarizations and intracellular Ca^{2+} elevations in the same OA interneuron as in A_3 after 45 min of washout of ryanodine. *B*: summary histograms for all cells tested with thapsigargin (open bars), ryanodine (shaded bar), or control cells (filled bars, in TTX only). Both the membrane depolarizations (*right graph*) and the Ca^{2+} response (area under curve, *left graph*) were significantly reduced compared with control cells ($^{**}P < 0.05$, analysis of variance).

values observed in TTX (data not shown). However, bath application of 10 μM ryanodine for 30 min significantly reduced 1S,3R-ACPD responses in four cells. The 1S,3R-ACPD evoked intracellular Ca^{2+} rise was completely blocked in three of four cells (e.g., Fig. 7A_3; 391 ± 391% $\Delta F/F \times$ s, $n = 4$ cells), and the membrane depolarization significantly reduced (5.8 ± 4.1 mV, $n = 4$ cells; Fig. 7). Washout of ryanodine was difficult to achieve given the long period of ryanodine exposure, although on reperfusion with standard aCSF 1S,3R-ACPD evoked a Ca^{2+} response in two of four cells (e.g., Fig. 7A_4) and a depolarizing response (peak depolarization 14.4 ± 3.9 mV) in three of four cells. Overall, these data suggest that ryanodine-sensitive intracellular Ca^{2+} stores are required for the 1S,3R-ACPD oscillatory responses.

1S,3R-ACPD oscillatory responses are specific to OA interneurons

Different types of interneurons have functionally distinct roles in the hippocampus (Freund and Buzsáki 1996). Almost all interneurons in OA tested (66/68) responded to 1S,3R-ACPD with large membrane depolarizations and intracellular Ca^{2+} rises as described. We did not find heterogeneous populations of cells in OA responsive and unresponsive to 1S,3R-ACPD as previously described by McBain et al. (1994), but there was a large range of depolarizing responses to 1S,3R-ACPD (11.8–40.0 mV). To examine if the oscillations in membrane potential and intracellular Ca^{2+} elicited by mGluR activation were specific to certain interneuron subtypes, we assessed 1S,3R-ACPD responses with a similar experimental protocol in interneurons located in or near stratum LM. Brief application (3–5 min) of 1S,3R-ACPD in the presence of TTX elicited significantly smaller membrane depolarization (5.6 ± 1.1 mV) and intracellular Ca^{2+} responses (3,953 ± 1,126% $\Delta F/F \times$ s) in LM cells (Fig. 8, $n = 11$). In all but one case, the depolarizing response to 1S,3R-ACPD in the presence of TTX was larger in OA than in LM interneurons. In this LM cell, 1S,3R-ACPD evoked a large depolarization (peak amplitude: 14.1 mV), but it was only accompanied by small intracellular Ca^{2+} elevation (area under Ca^{2+} response: 4,140% $\Delta F/F \times$ s).

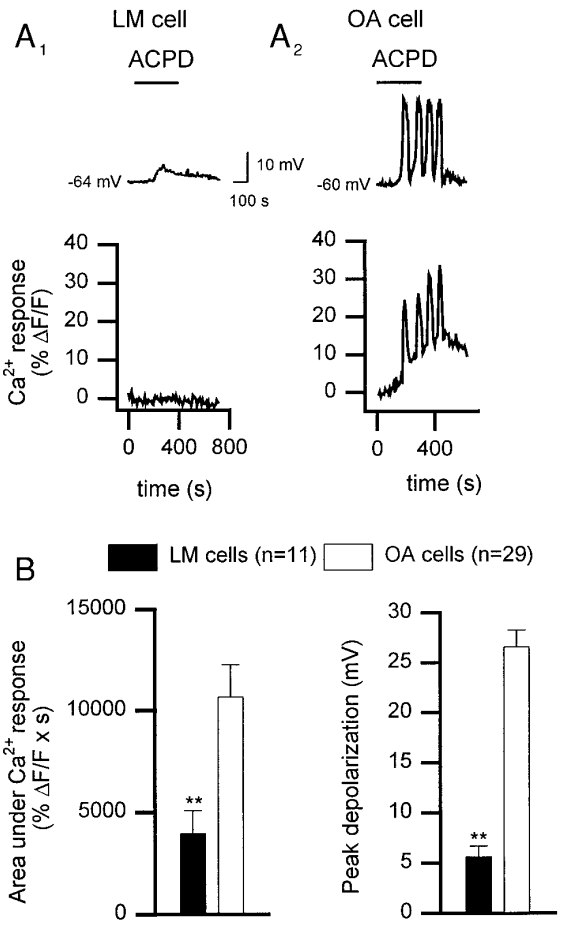

FIG. 8. Oscillatory 1S,3R-ACPD responses are found specifically in interneurons of OA and not in those located near stratum lacunosum-moleculare (LM). A_1: application of 1S,3R-ACPD (at time indicated by bar) in the presence of TTX evoked a small membrane depolarization (*top trace*) and no change in intracellular Ca^{2+} level (*bottom graph*) in a representative LM interneuron. A_2: in contrast, a similar application of 1S,3R-ACPD elicited oscillatory membrane depolarizations and intracellular Ca^{2+} rises in a typical OA cell. *B*: summary histograms for all cells tested in LM (filled bar) and OA (open bar) showing the significantly smaller depolarizing responses (*right graph*) and intracellular Ca^{2+} elevations (*left graph*) in cells of LM compared with those of cells in OA ($^{**}P < 0.05$, Student's *t*-test).

Morphology of biocytin-filled interneurons

Recordings were made with internal patch solution containing biocytin to identify morphologically the subtypes of interneurons that were selectively responsive to 1S,3R-ACPD. Representative examples of biocytin-filled interneurons located in OA and LM are illustrated in Fig. 9. Biocytin-filled interneurons in OA that showed pronounced 1S,3R-ACPD responses had multipolar soma and horizontally oriented dendrites in OA ($n = 8$ cells; e.g., Fig. 9*A*). In addition, some of these OA cells had vertically oriented dendrites crossing stratum pyramidale and extending across stratum radiatum ($n = 4$ cells). The axon of OA cells coursed either only in OA (2 cells) or also in stratum radiatum and LM (5 cells). OA interneurons with horizontally oriented dendrites in OA and with axon projections in LM (McBain et al. 1994) or with vertically oriented dendrites in stratum radiatum (Lacaille et al. 1987) exhibited similar responses to 1S,3R-ACPD. In contrast, biocytin-labeled LM interneurons that did not show large oscillatory 1S,3R-ACPD responses had multipolar soma and radially oriented dendrites in stratum radiatum and LM ($n = 9$ cells; e.g., Fig. 9*B*). The axon of LM cells arborized in stratum radiatum and LM (7 cells) and occasionally also coursed through stratum pyramidale and into stratum oriens (2 cells).

DISCUSSION

Our principal findings were that activation of group I/II mGluRs by 1S,3R-ACPD generated oscillatory membrane depolarizations and intracellular Ca^{2+} elevations in interneurons of OA because of a functional coupling between voltage-dependent Ca^{2+} conductances and Ca^{2+} release from ryanodine-sensitive intracellular stores. These oscillatory responses were specific to certain subtypes of interneurons because 1S,3R-ACPD produced only low amplitude depolarizations and small rises in intracellular Ca^{2+} in other interneurons located near LM.

Our results confirm a previous report of mGluR-induced oscillatory membrane currents in OA interneurons (McBain et al. 1994) but in addition show that these currents are associated with large increases in intracellular Ca^{2+} levels involving both Ca^{2+} entry via voltage-dependent Ca^{2+} channels and release from intracellular stores. We also found that the majority of OA interneurons were responsive to 1S,3R-ACPD. A previous report indicated that a specific subtype of OA interneurons, horizontal cells, preferentially displayed this oscillatory mGluR response (McBain et al. 1994). Our morphological results indicate that two subtypes of OA interneurons, horizontal and vertical (Lacaille and Williams 1990) cells, show these mGluR responses but that other types of interneurons we examined in or near LM do not. Therefore mGluR activation differentially affects interneuron subtypes and may influence hippocampal function in multiple, interneuron-specific ways. The apparent discrepancy with the previous report may be due to age differences of animals used (19–24 vs. 14–21 days in our study) because 1S,3R-ACPD concentration and rat species were similar in both studies. mGluR expression reaches a peak at postnatal days 7–10 in CA1 hippocampus and declines thereafter toward a nadir at day 24 (Nicoletti et al. 1986). Thus developmental down-regulation of mGluRs in certain subtypes of interneuron may account for the observed differences in 1S,3R-ACPD responsitivity.

Group I/II mGluRs and ryanodine-sensitive intracellular stores mediate oscillatory responses

The block of oscillatory membrane depolarizations and Ca^{2+} responses by the antagonist S-MCPG indicates that group I/II mGluRs are involved. These results are consistent with reports of high level of expression of mGluR1α in OA interneurons (Baude et al. 1993; Masu et al. 1991). The oscillatory responses may therefore be mediated by the activation of the mGluR1α subtype. The block of oscillatory membrane depolarization and Ca^{2+} rise by either thapsigargin, which blocks the Ca^{2+}-ATPase (Sagara et al. 1992) and depletes both IP_3- and ryanodine-sensitive stores, or ryanodine, which depletes stores linked to ryanodine recep-

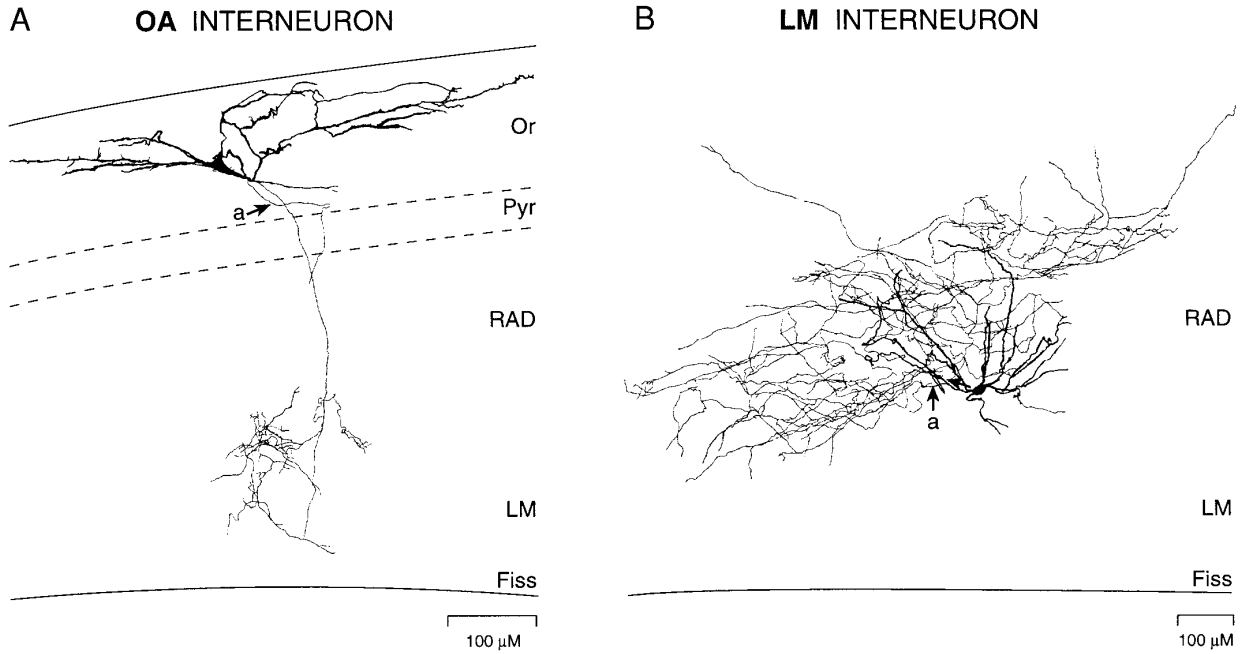

FIG. 9. Morphological characterization of interneurons responsive to 1S,3R-ACPD. Representative examples of biocytin-filled interneurons in OA (*A*) and near LM (*B*). The OA interneuron had horizontally oriented dendrites in stratum oriens and an axon that projected to LM. The LM interneuron had multiple primary dendrites that extended radially in stratum radiatum and an axon that projected profusely in stratum radiatum and that extended a few collaterals in stratum pyramidale and in LM.

tors (Smith et al. 1988), suggests that ACPD responses require the release of Ca^{2+} from intracellular stores sensitive to ryanodine but not to IP_3. Our observations are consistent with the inverse correlation reported between mGluR1α mRNA levels and 1S,3R-ACPD-stimulated polyphosphoinositide turnover in many brain regions (Condorelli et al. 1992) and between mGluR1 and IP_3-R distribution in stratum oriens of the hippocampus (Fotuhi et al. 1993). Because mGluR1α is the predominant mGluR receptor subtype in OA interneurons (Baude et al. 1993), oscillatory membrane depolarizations and Ca^{2+} responses in OA interneurons may be linked to a second messenger system other than IP_3. In this area, mGluR1α and phorbol ester binding sites are correlated, suggesting that mGluR actions may be mediated by the protein kinase C pathway of the phosphoinositide system (Fotuhi et al. 1993). Because ryanodine effectively blocked oscillatory responses to 1S,3R-ACPD, mGluR1α activation may thus be coupled to protein kinase C stimulation and to Ca^{2+}-induced Ca^{2+} release via ryanodine receptors in OA interneurons.

Interaction between Ca^{2+} channels and Ry-Rs during 1S,3R-ACPD induced oscillatory responses

The requirement for both Ca^{2+} entry via voltage-dependent Ca^{2+} channels and release from intracellular Ca^{2+} stores suggests a functional coupling, induced by mGluR activation, between Ca^{2+} channels and intracellular ryanodine receptors. Enhancement of Ca^{2+} influx through voltage-gated channels by 1S,3R-ACPD was reported in septal neurons; however, Ca^{2+} release from internal stores was not involved (Zheng et al. 1996). Chavis et al. (1996) described 1S,3R-ACPD–mediated activation of mGluR1s in cerebellar granule cells, which triggered an oscillatory, facilitatory interaction between RyRs and L-type Ca^{2+} channels via the production of a second messenger different from IP_3. Consistent with this, we hypothesized that 1S,3R-ACPD facilitated voltage-gated Ca^{2+} influx and Ca^{2+}-induced Ca^{2+} release via Ry-Rs, leading to oscillations in intracellular Ca^{2+} in OA interneurons (Fig. 10). In this scheme, the link between mGluR1 stimulation and activation of voltage-gated Ca^{2+} channels remains to be determined as well as the mechanisms responsible for the periodicity of oscillations. The Ca^{2+} increase could be curtailed by Ca^{2+}-dependent inhibition of Ry-R mediated release, and subsequent sequestration of internal Ca^{2+} may reset the system for another Ca^{2+} event (Henzi and MacDermott 1992). In this model, blockade of either Ca^{2+} entry or release from internal stores prevents oscillations because both are necessary for this functional interaction.

Currents underlying membrane depolarizations induced by mGluRs

Membrane depolarizations and Ca^{2+} rises evoked by 1S,3R-ACPD persisted in TTX and were blocked by extracellular Cd^{2+} or removal of extracellular Ca^{2+}, indicating that Ca^{2+} but not Na^+ entry through voltage-gated channels was necessary in generating oscillatory responses. It was previously reported that mGluR-induced oscillatory membrane currents were dependent on intracellular Ca^{2+} but insensitive to extracellular Co^{2+}, suggesting that they were due to a Na^+/Ca^{2+} exchanger (McBain et al. 1994). Alternatively, the current responsible for the depolarization plateau accompanying the Ca^{2+} rise could be a Ca^{2+}-activated nonspecific cation current (CAN current) (Crépel et al.

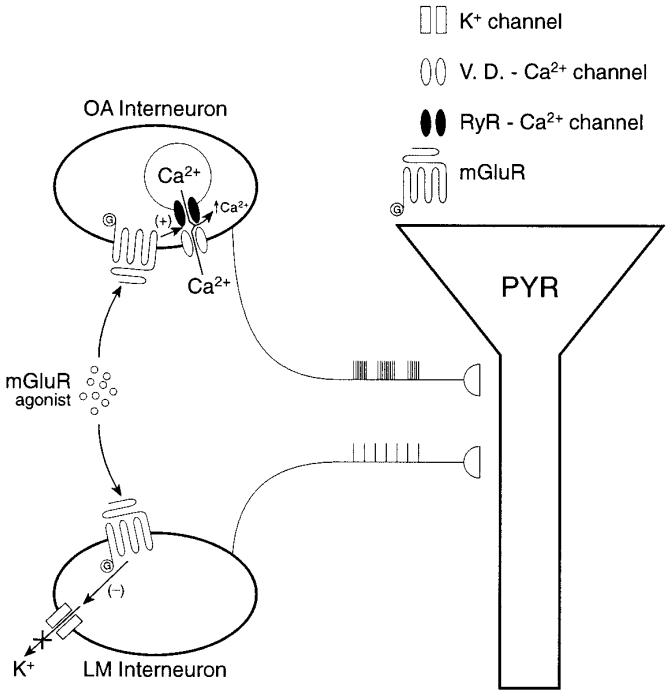

FIG. 10. Diagram of interneuron-specific mGluR actions. mGluR activation leads to a negative modulation of K^+ currents, resulting in a small, sustained depolarization of interneurons of LM. However, in OA interneurons, mGluR activation leads to a functional coupling between voltage-dependent Ca^{2+} channels and ryanodine-sensitive intracellular Ca^{2+} stores, producing repetitive elevations in intracellular Ca^{2+} levels and membrane depolarizations. The latter effect results in repetitive burst firing in OA interneurons, whereas the former results in tonic discharges from LM interneurons.

1994; Partridge and Swandulla 1988). The slow time course of the membrane depolarization and Ca^{2+} response is consistent with a mechanism involving the Na^+/Ca^{2+} exchanger, and we attempted to block it by substituting extracellular Na^+ with Li^+ (Crépel et al. 1994; Keele et al. 1997). We found that aCSF containing LiCl was detrimental to cells and that even in the presence of TTX membrane instability in LiCl precluded adequate electrophysiological recording (see also McBain et al. 1994). Keele et al. (1997) reported that 1S,3R-ACPD activated a Na^+/Ca^{2+} exchanger in amygdala neurons, but this Li^+-sensitive exchange current was only revealed in the presence of potassium channels blockers Cs^+ or Ba^{2+}. Because oscillatory responses in OA interneurons were sensitive to manipulations affecting either internal stores or Ca^{2+} entry, and recorded without K^+ channel blockers, a CAN current seems more likely to be responsible for these depolarizations. In CA1 pyramidal cells, 1S,3R-ACPD-induced CAN currents are similarly sensitive to Cd^{2+} and low extracellular Ca^{2+} (Crépel et al. 1994).

Physiological implications

1S,3R-ACPD application likely activated a large number of mGluRs on individual interneurons, and whether similar mGluR-mediated oscillatory responses can be elicited by synaptic activation remains unknown. Batchelor and Garthwaite (1997) reported synaptically evoked mGluR responses at parallel-fiber synapses in cerebellar Purkinje cells. In preliminary experiments, we were not able to elicit similar oscillatory membrane depolarizations and Ca^{2+} responses in interneurons with electrical stimulation of afferents (Ouardouz, Woodhall, and Lacaille, unpublished observations). However, in the elevated K^+ model of epilepsy, the activity of interneurons in OA shifted from a tonic to phasic firing pattern caused by the activation of a periodic inward current that was MCPG sensitive (McBain 1994). Thus, even if stimulus-evoked synaptic activity can elicit mGluR-mediated events in some central neurons, much more intense synaptic stimulation may be required to activate mGluR responses in OA interneurons. Such intense levels of synaptic activity, however, may be found in pathological conditions such as epileptic seizures. The selective activation of mGluR oscillatory responses in OA interneurons and the prolonged nature of the evoked Ca^{2+} responses suggest that these mGluR mechanisms may play a role in the selective vulnerability of OA interneurons to excitotoxicity (Best et al. 1993; Houser and Esclapez 1996; Morin et al. 1998). In some OA cells, mGluR-evoked Ca^{2+} rises did not return to baseline even after prolonged 1S,3R-ACPD washout (e.g., Figs. 1 and 2). Because series resistance was always $\leq$30 MΩ, and 20 min was allowed for dye loading at the beginning of each experiment, it seems unlikely that ongoing dye loading could explain this increase in basal levels. Alternatively, some OA cells may have experienced an intracellular Ca^{2+} load induced by 1S,3R-ACPD, even if electrophysiological recordings returned to baseline levels. Khodorov et al. (1996) reported that mitochondrial deenergization after prolonged GluR activation can lead to a Ca^{2+} overload in neurons. Additionally, in some OA interneurons excluded from this study ($n = 33$), oscillatory intracellular Ca^{2+} responses were superimposed on a persistent increase in basal Ca^{2+} levels, associated with a deterioration of electrophysiological recordings. Before 1S,3R-ACPD application, all cells showed normal electrophysiological responses. Thus, during epileptic seizures, OA interneurons may experience a prolonged activation of mGluRs and large increases in Ca^{2+} levels, which may lead to excitotoxic processes selectively in those interneurons (McDonald et al. 1993; Olney 1994; Taylor et al. 1995). Thus interneuron-specific mGluR actions may have important implications for normal and pathological hippocampal function.

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