Group I mGluR Activation Causes Voltage-Dependent and -Independent Ca$^{2+}$ Rises in Hippocampal Pyramidal Cells

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Bianchi, Riccardo, Steven Young, and Robert K. S. Wong. Group I mGluR activation causes voltage-dependent and -independent Ca$^{2+}$ rises in hippocampal pyramidal cells. J. Neurophysiol. 81: 2903–2913, 1999. Application of the metabotropic glutamate receptor (mGluR) agonist (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid (ACPD) or the selective group I mGluR agonist (S)-3,5-dihydroxyphenylglycine (DHPG) depolarized both CA3 and CA1 pyramidal cells in guinea pig hippocampal slices. Simultaneous recordings of voltage and intracellular Ca$^{2+}$ levels revealed that the depolarization was accompanied by a biphasic elevation of intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]i); a transient calcium rise followed by a delayed, sustained elevation. The transient [Ca$^{2+}$]i rise was independent of the membrane potential and was blocked when caffeine was added to the perfusing solution. The sustained [Ca$^{2+}$]i rise, appeared when membrane depolarization reached threshold for voltage-gated Ca$^{2+}$ influx and was suppressed by membrane hyperpolarization. The depolarization was associated with an increased input resistance and persisted when either the transient or sustained [Ca$^{2+}$]i responses was blocked. mGluR-mediated voltage and [Ca$^{2+}$]i responses were blocked by (+)-α-methyl-4-carboxyphenylglycine (MCPG) or (S)-4-carboxy-3-hydroxyphenylglycine (4CHPG). These data suggest that in both CA3 and CA1 hippocampal cells, activation of group I mGluRs produced a biphasic accumulation of [Ca$^{2+}$]i via two paths: a transient release from intracellular stores, and subsequently, by influx through voltage-gated Ca$^{2+}$ channels. The concurrent mGluR-induced membrane depolarization was not caused by the [Ca$^{2+}$]i rise.

INTRODUCTION

Recent studies show that metabotropic glutamate receptor (mGluR) activation elicits hyperpolarization (Jaffe and Brown 1994; Shirasaki et al. 1994) and/or depolarization in hippocampal pyramidal cells (Charpak et al. 1990; Congar et al. 1997; Crépel et al. 1994; Guérineau et al. 1994, 1995; Pozzo Miller et al. 1995, 1996; Shirasaki et al. 1994). There has been considerable interest in elucidating the second-messenger mechanisms for the mGluR-induced membrane responses. Initial studies indicate the involvement of G-proteins in these responses (Congar et al. 1997; Pozzo Miller et al. 1995; Shirasaki et al. 1994; but see Guérineau et al. 1995). In particular, the role of intracellular Ca$^{2+}$ in mediating mGluR-induced membrane responses has been evaluated, because this ion is a second messenger for one class of mGluRs (see below). One study reports that intracellular Ca$^{2+}$ directly sustains an mGluR-mediated depolarization by activating a nonspecific cation current in CA1 hippocampal neurons (Congar et al. 1997). However, other studies performed in CA3 pyramidal cells suggest that mGluR-induced depolarizations do not require an intracellular Ca$^{2+}$ rise (Guérineau et al. 1995; Pozzo Miller et al. 1995). In CA1 neurons it has been suggested that transient intracellular Ca$^{2+}$ increases induced by mGluR agonists elicit hyperpolarizing currents (Jaffe and Brown 1994; Shirasaki et al. 1994). It is possible that these different findings reflect the existence of multiple components of mGluR-induced membrane responses that are differentially expressed in CA1 and CA3 neurons. In an attempt to clarify the causal relationship between intracellular Ca$^{2+}$ and voltage responses to mGluR stimulation, we simultaneously recorded intracellular Ca$^{2+}$ levels ([Ca$^{2+}$]i) and membrane potential in hippocampal cells.

Elevation of [Ca$^{2+}$]i following mGluR activation may occur via release from intracellular stores (Pozzo Miller et al. 1996) or through transmembrane influx. Stimulation of group I mGluRs activates phosphoinositide hydrolysis and secondarily elevates [Ca$^{2+}$]i, by inducing release from inositol-1,4,5-trisphosphate (IP$_3$)-sensitive stores (Abe et al. 1992; Masu et al. 1991). Transmembrane influxes of Ca$^{2+}$ may also contribute to the mGluR-induced [Ca$^{2+}$]i rise. This influx can occur via nonspecific cation channels (Chen et al. 1997; Partridge et al. 1994) or voltage-gated Ca$^{2+}$ channels.

By monitoring both the voltage and intracellular Ca$^{2+}$ responses in single CA3 and CA1 pyramidal cells, we were able to address two questions: 1) What are the origin and temporal pattern of [Ca$^{2+}$]i rise elicited by mGluR agonists? 2) What is the causal relationship between the [Ca$^{2+}$]i rise and mGluR-induced membrane potential changes?

Parts of this study have been presented in abstract form (Bianchi et al. 1997; Young et al. 1996).

METHODS

Slice preparation

Transverse hippocampal slices (~300 μm thick) were prepared from adult guinea pigs as described previously (Bianchi and Wong 1995). Slices were placed submerged in a coverslip-bottomed recording chamber and superfused at 3–5 ml/min at 30–32°C. The composition of the perfusing control solution was (in mM) 124 NaCl, 26 NaHCO$_3$, 5 KCl, 1.6 MgCl$_2$, 2.0 CaCl$_2$, and 10 d-glucose. The solution was continuously gassed with a 95% O$_2$-5% CO$_2$ mixture (pH 7.4). One slice at a time was placed in the chamber and was held down by nylon threads glued to a platinum ring. The arrangement provided sufficient mechanical stability to allow extended intracellular recordings but did not prevent solution exchange at the bottom surface of the slice. The chamber was mounted on the stage of a Nikon Diaphot inverted microscope. Optical access to the slice was through the glass coverslip at the bottom of the chamber.

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Optical recordings

Intracellular recordings were performed in the pyramidal cell layer of the CA3 and CA1 regions with sharp glass electrodes containing potassium acetate (0.4 M) and calcium green-1 (0.5 mM). In some experiments the quaternary lidocaine derivative N-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX-314; 50 mM) was added to the electrode-filling solution. The electrode resistance was typically 50–80 MΩ. Recordings in current-clamp mode were amplified (Axoclamp-2A; Axon Instruments, Foster City, CA), displayed on an oscilloscope (DSO 400; Gould, Valley View, OH) and chart recorder (Gould TA240), and stored on FM tape (Racal Store 4DS; Southampton, England). When recorded concurrently with synchronized optical recordings (see Optical recordings), membrane voltage signals were filtered at 30–120 Hz for slow events (e.g., responses to (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD)) or at 1,500 Hz for faster events (e.g., responses to intracellular current pulse injections), and stored directly in a computer. Intracellular square-wave current pulses were timed by a digital stimulator (PG 4000; Neuro Data Instruments, New York, NY). Hyperpolarizing pulses (−0.1 to −1.0 nA, 150–300 ms) were applied throughout an experiment to monitor the condition of the cell membrane and to adjust the bridge balance (e.g., Fig. 12A).

Optical recordings

Changes in [Ca2+], were detected by filling cells with calcium green-1 (0.5 mM; C3010, Molecular Probes, Eugene, OR) from the intracellular recording electrodes. The dye was injected by passing 350-ns, −0.5-nA current pulses at 50% duty cycle for 20–25 min. Epi-illumination was provided by a 150 W xenon short gap bulb (XBO 150W CR ORF; Osram, Germany) linked to the microscope by a fused silica fiberoptic bundle (77578; Oriel, Stratford, CT). Filled cells were viewed through a Nikon ×10 Fluor objective (n.a. = 0.5) and standard fluorescein filter set (Nikon B-2E). The fluorescent images shown in Figs. 1A and 2B were divided into three and four areas, respectively, and gray-level scales were independently adjusted for the different regions. This was necessary to display both dendrites and soma, which had widely different fluorescence intensities. The computer program used for simultaneous acquisition of calcium green-1 fluorescence and transmembrane potential has been described previously (Lasser-Ross et al. 1991).

Fluorescence images were acquired from a thermoelectrically cooled charge-coupled device camera (CH230; Photometrics, Tucson, AZ) run in frame-transfer mode. A software-driven timer/pulser (Master-8; AMPI, Israel) provided for precise synchronization of optical and electrical recordings. Fluorescence values were measured and averaged over an area of interest selected within the fluorescence image. Relative intracellular Ca2+ levels in the areas of interest were expressed as change in fluorescence divided by the resting fluorescence (∆F/∆F₀). The applied equation was ∆F/∆F₀ (%) = 100 × (F − F₀)/(F₀ − F)., where F is the average fluorescence of the area of interest in each image, F₀ is the baseline fluorescence of the area of interest averaged over at least 10 images before stimulation, and F₀ is the autofluorescence measured at an equivalent location in the slice at which no stimulus-associated signal was detected. Normalized in this way, the dye fluorescence is a monotonic function of the concentration of intracellular Ca2+ (Callaway et al. 1993). To reduce bleaching and phototoxic damage to the cell, neutral density filters were introduced in the excitation light path. In a few cases (n = 6 of 76 cells), traces were corrected for bleaching (2–7% of baseline fluorescence over 5–6 min) by subtracting a recording made without stimulation (e.g., Fig. 6B). Cells were accepted for optical recordings only when their fluorescence responses (∆F/∆F₀) to brief trains of action potentials were >10).

Consistent with previous reports, action potentials elicited Ca2+ increases that were substantially larger in the proximal apical and basal dendrites than in somata (Fig. 2) (cf. Jaffe et al. 1994; Regehr and Tank 1992). Due to light scattering from intervening tissue, most of the cells we recorded were too deep within a slice to clearly distinguish dendritic signals. Thus, in this paper we report Ca2+ signals measured in somata (except for Fig. 2). In dye-filled cells near the slice surface, however, dendrites were clearly visualized, and dendritic responses to ACPD were also apparent (e.g., Fig. 2).

Pharmacological agents

Agonists of mGluRs were injected directly into the bath via a narrow tube from a syringe (pulse application). A pulse application lasted on average 7.1 ± 0.6 s (mean ± SE; range, 2.5–12.0 s; n = 20). Antagonists of mGluRs were added to the perfusate (bath application). For pulse applications, 100 μl of 2 mM ACPD (or 1 mM DHPG) was typically used. We attempted to characterize the time course of the agonist concentration after a pulse injection by applying 3 M KCl via the syringe tubing and monitoring the junction potential change in the recording pipette. The average time-to-peak of the potential change
was 19.1 ± 1.2 s (range, 7–29 s) from the start of the injection and the half-amplitude duration (see Data analysis) was 208.5 ± 25.4 s (range, 23–487 s; n = 17). During the time required to reach peak agonist concentration at the cell (7–29 s), the bolus of 100 μl of 2 mM ACPD was diluted by perfusing solution that continued to flow into the chamber. The dilution can be estimated by considering the volume of the bath (0.9–1 ml) and a volume of solution of 0.3–2.4 ml perfusing in 7–29 s (at a flow rate of 3–5 ml/min), and yielded peak ACPD concentrations of 50–150 μM (25–75 μM for DHPG). Caffeine was either bath-applied (2–5 mM; e.g., Fig. 8, A and B) or pulse-applied (estimated concentration, 2–5 mM; e.g., Fig. 8C). Agonist applications to the same cell were separated by intervals of 30–40 min to allow complete recovery of the responses. In most experiments tetrodotoxin (0.3–0.6 mM) was present in the perfusate during the recordings.

ACPD (50–150 μM), (S)-3,5-dihydroxyphenylglycine (DHPG; 25–75 μM), (+)-α-methyl-4-carboxyphenylglycine (MCPG; 0.5–1 mM), and (S)-4-carboxy-3-hydroxyphenylglycine (4C3HPG; 0.5 mM) were purchased from Tocris Cookson (Ballwin, MO); QX-314 was obtained from Research Biochemical International (Natick, MA); all the other chemicals were from Sigma (St. Louis, MO).

Data analysis

Data were analyzed using pClamp (Axon Instruments), Transform (Fortner Research LLC, Sterling, VA), and SigmaPlot (SPSS, Chicago, IL) software. Figures 1A and 2A are montages of different display scales applied to single images of CA3 pyramidal neurons filled with calcium green-1 (Photoshop, Adobe Systems, San Jose, CA). This is required for the display of unnormalized images because of the vastly greater dye-containing volume of the cell body compared with the dendrites.

Input resistance ($R_{in}$) was calculated from the amplitudes of the voltage responses divided by the intensity values of the injected
current steps. In five cells (Fig. 9B), \( R_{in} \) was calculated as the slope of voltage-current (\( V-I \)) plots obtained from responses to depolarizing ramp current injections. Half-amplitude durations were measured from the time the rising phase of a response crossed one half the peak amplitude until the decay phase crossed the same line. Measured parameters are reported as means \( \pm \) SE. Student’s \( t \)-tests were applied for statistical comparisons, and differences were considered significant when \( P < 0.05 \).

**RESULTS**

Intracellular recordings were performed on 73 CA3 and 20 CA1 pyramidal neurons, all with overshooting action potentials. The average resting membrane potential (\( V_{rest} \)) and input resistance (\( R_{in} \)) of CA3 cells were \(-62.7 \pm 0.7 \) (SE) mV and \( 49.8 \pm 2.5 \) M\( \Omega \), respectively. In CA1 cells \( V_{rest} \) was \(-63.3 \pm 1.1 \) mV and \( R_{in} \) was \( 36.2 \pm 2.4 \) M\( \Omega \). Of all neurons, 60 CA3 and 16 CA1 cells were successfully injected with the fluorescent dye calcium green-1 for simultaneous electrical and optical recordings (see Fig. 1). In general, CA1 cells responded to mGluR agonists in the same way as CA3 cells, and the main conclusions of this paper apply to both regions. Where regional differences were observed, they will be illustrated in the section on mGluR responses in CA1.

**ACPD-induced intracellular voltage and calcium responses in CA3 neurons**

Membrane potential and intracellular \( \text{Ca}^{2+} \) responses were simultaneously recorded in CA3 cells from slices superfused with control solution (\( n = 6 \)). Application of ACPD (50–150 \( \mu \text{M} \); \( n = 12 \)) elicited depolarization and action potentials (Fig. 1). Following an initial phase of intense firing, action potentials decreased in frequency and the membrane potential gradually recovered to the resting level.

![FIG. 3. Voltage-sensitive and voltage-insensitive components of the ACPD-induced \([\text{Ca}^{2+}]_i\) response. A: a CA3 pyramidal cell was hyperpolarized to \(-74 \) mV and a depolarizing ramp current (\( I \)) was manually applied. The neuron was then returned to \(-74 \) mV, and ACPD was applied. Hyperpolarizing pulses (\(-0.1 \) nA, 4 s, 1 every 24 s) were applied during the ramp and the initial ACPD-induced depolarization. The period between the 2 arrows is expanded in B, accompanied by the corresponding optical responses (\([\text{Ca}^{2+}]_i\)). B: a depolarizing ramp was used to estimate the threshold for voltage-dependent \( \text{Ca}^{2+} \) influx (\(-60 \) mV; horizontal dotted line). At membrane potentials above this value the \([\text{Ca}^{2+}]_i\) increase was transiently suppressed by hyperpolarizing pulses (just after the left vertical dotted line). During the ACPD response hyperpolarizing pulses did not affect the transient \([\text{Ca}^{2+}]_i\) increase (arrow) but did decrease the later sustained \([\text{Ca}^{2+}]_i\) elevation (after the right vertical dotted line). Note that the \([\text{Ca}^{2+}]_i\) rise during the sustained phase of the ACPD response occurred above the voltage threshold for \( \text{Ca}^{2+} \) entry estimated during the ramp (horizontal dotted line).](http://jn.physiology.org/)

In five cells (Fig. 9B), \( R_{in} \) was calculated as the slope of voltage-current (\( V-I \)) plots obtained from responses to depolarizing ramp current injections. Half-amplitude durations were measured from the time the rising phase of a response crossed one half the peak amplitude until the decay phase crossed the same line. Measured parameters are reported as means \( \pm \) SE. Student’s \( t \)-tests were applied for statistical comparisons, and differences were considered significant when \( P < 0.05 \).

![FIG. 4. Effects of steady hyperpolarization on the time course of the ACPD-induced \([\text{Ca}^{2+}]_i\) responses. ACPD was consecutively applied to the same cell at rest (A) and at 3 hyperpolarized membrane potentials (hyperpolarized by means of DC current injections: \(-0.16 \) nA in B, \(-0.7 \) nA in C, and \(-1.35 \) nA in D). In A–C, ACPD induced a gradual depolarization that triggered \( \text{Ca}^{2+} \) spikes (\( V_m \)) and sustained \([\text{Ca}^{2+}]_i\) elevations (\( \text{Ca}^{2+} \)) and spiked. The membrane hyperpolarization delayed the \( \text{Ca}^{2+} \) spikes and the sustained component of the \([\text{Ca}^{2+}]_i\) response, revealing an initial transient rise of \([\text{Ca}^{2+}]_i\) (arrows in B–D). In D, the steady hyperpolarization prevented spike firing and further delayed the depolarization, whereas the initial transient \([\text{Ca}^{2+}]_i\) rise was not significantly altered. Following subsequent ACPD applications (at \(-75 \) and \(-64 \) mV; not shown), depolarization, spike firing, and sustained \([\text{Ca}^{2+}]_i\) elevations reappeared, similar to the responses in C and B, respectively.](http://jn.physiology.org/)
Concurrent fluorescence measurements from the soma showed that ACPD applications produced an abrupt increase in the fluorescence of calcium green-1, indicating an increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)). Brief increases in the Ca\(^{2+}\) signal occurred in phase with action potential firing (Fig. 1). In addition, there was a baseline increase in [Ca\(^{2+}\)]\(_i\) with a time course parallel to that of the depolarization.

Under control conditions, both the optical and voltage records of the ACPD-induced response were clearly dominated by action potentials. To examine the voltage and [Ca\(^{2+}\)]\(_i\) responses directly elicited by mGluR activation, tetrodotoxin (TTX, 0.3–0.6 \(\mu\)M) was added to the perfusing solution in all remaining experiments. TTX suppressed fast Na\(^{+}\)-mediated action potentials and their associated voltage-dependent Ca\(^{2+}\) increases. Under these conditions, ACPD induced a more gradual depolarization and increase in [Ca\(^{2+}\)]\(_i\) (Fig. 2B). The increase in [Ca\(^{2+}\)]\(_i\) often occurred in two phases: an initial transient phase and a later sustained phase. The transient phase consisted of a rapid rise that peaked before the voltage response (Fig. 2R, arrow). On average, the transient Ca\(^{2+}\) component peaked at 31.3 ± 3.6 s after ACPD application (n = 25), whereas the depolarization peaked at 118.6 ± 9.5 s (n = 25). The transient Ca\(^{2+}\) component was followed by a sustained phase of Ca\(^{2+}\) rise (Fig. 2B). The depolarization and the sustained Ca\(^{2+}\) response returned to baseline levels in 5–20 min (n = 4).

ACPD-induced [Ca\(^{2+}\)]\(_i\) increases were not restricted to the soma. In cells where Ca\(^{2+}\) responses could be visualized in the dendrites (Fig. 2, C and D), the biphasic [Ca\(^{2+}\)]\(_i\) response induced by ACPD was observed both in the soma and in the proximal apical and basal dendrites (Fig. 2, A and B).

![Figure 5](image-url)  
**FIG. 5.** Time course of the isolated transient Ca\(^{2+}\) response. A: intracellular Ca\(^{2+}\) (Ca\(^{2+}\)) and voltage responses (V\(_m\)) to ACPD recorded in a CA3 neuron. Initial membrane potential, −78 mV. Peak transient [Ca\(^{2+}\)]\(_i\) response (arrow) occurred at 14 s and returned to baseline at 57 s; half-amplitude duration, 22 s. The duration of the transient Ca\(^{2+}\) rise was significantly shorter than the wash out time of high K\(^+\) applications to the bath (26.8 ± 2.6 s, see RESULTS, vs. 208.5 ± 25.4 s, see METHODS; \(P < 0.001\)), suggesting that the time course of the transient Ca\(^{2+}\) response is not determined by that of the drug concentration in the bath. B: average fluorescence (○) and voltage changes (●) following ACPD application (n = 5 cells). Traces were sampled at 1 Hz, and the different applications were aligned at the onset of the [Ca\(^{2+}\)]\(_i\) response. Error bars are means ± SE. The average membrane potential before ACPD application was −68.6 ± 0.9 mV. Note that the membrane voltage depolarization outlasts the [Ca\(^{2+}\)]\(_i\) response (arrow).

**FIG. 6.** Effects of the mGluR antagonist (+)-α-methyl-4-carboxyphenylglycine (MCPG) on the ACPD-induced Ca\(^{2+}\) and voltage responses. Records from the same CA3 pyramidal cell in control conditions (A) and in the presence of MCPG (1 mM) in the perfusing solution (B). A current ramp (I) was injected before the application of ACPD to depolarize the cell from its resting membrane potential (−61 mV) and to induce a voltage-dependent [Ca\(^{2+}\)]\(_i\) increase. The membrane potential was then returned to rest before ACPD application. Fast downward deflections (in V\(_m\) and I, and in Figs. 9A and 10) are hyperpolarizing steps used to monitor input resistance (\(R_{\text{in}}\)) and bridge balance. The mGluR antagonist MCPG suppressed the ACPD-induced Ca\(^{2+}\) (Ca\(^{2+}\)) and voltage (V\(_m\)) responses but not the Ca\(^{2+}\) rise induced by the depolarizing ramp. Ca\(^{2+}\) traces were corrected for bleaching. The decline in fluorescence due to bleaching (B, inset: top trace) was subtracted from the experimental trace (B, inset: bottom trace; see METHODS). This example shows the maximum extent of bleaching (−7% of the baseline fluorescence over 5 min) that occurred among the data in this study.

**Characteristics of the biphasic [Ca\(^{2+}\)]\(_i\) rise**

To identify the sources of [Ca\(^{2+}\)]\(_i\) rise, we tested the voltage sensitivity of the ACPD-induced Ca\(^{2+}\) responses (Fig. 3). The threshold for voltage-gated Ca\(^{2+}\) influx, as shown by increase in calcium green-1 fluorescence, was determined by applying a depolarizing current to the cell. The mean threshold for Ca\(^{2+}\) influx was −60.3 ± 0.7 mV (n = 29 cells; Fig. 3B, left vertical dotted line). Intracellular Ca\(^{2+}\) levels were transiently depressed by hyperpolarizing pulses applied at membrane potentials above the threshold for Ca\(^{2+}\) entry.

After determining the threshold for voltage-gated Ca\(^{2+}\) entry, ACPD was applied to the slice. ACPD elicited a depolarization (Fig. 3, A and B, V\(_m\)) and a biphasic [Ca\(^{2+}\)]\(_i\) increase (Fig. 3B: Ca\(^{2+}\)). Hyperpolarizing pulses applied via the recording electrode affected the two phases of the Ca\(^{2+}\) response differently: the [Ca\(^{2+}\)]\(_i\) during the early transient phase was not
affected, whereas the [Ca\(^{2+}\)] during the second phase was suppressed.

Comparison of the responses induced by current injection with those induced by ACPD showed that the initial transient Ca\(^{2+}\) rise occurred subthreshold to voltage-gated Ca\(^{2+}\) entry (below the horizontal dotted line in Fig. 3B, \(V_m\)). In contrast, the sustained phase of Ca\(^{2+}\) rise occurred only at membrane potentials above threshold (horizontal dotted line in Fig. 3B, \(V_m\)).

**Time course of the initial transient [Ca\(^{2+}\)] rise**

Because the transient [Ca\(^{2+}\)] rise was not suppressed by membrane hyperpolarization, we isolated this component by applying ACPD at increasing levels of hyperpolarization. At resting membrane potentials, the ACPD-induced Ca\(^{2+}\) response in some cells consisted of a continuous increase instead of a biphasic response (Fig. 4A). The transient Ca\(^{2+}\) component was then revealed when the cell was hyperpolarized by current injection (Fig. 4B; arrow). Upon increasing the level of hyperpolarization, complete separation of the initial transient component from the sustained component of [Ca\(^{2+}\)] increase was observed (Fig. 4, C and D).

The time course of the isolated transient [Ca\(^{2+}\)] rise was analyzed in experiments in which ACPD was applied at hyperpolarized levels (Fig. 5; \(n = 5\)). The [Ca\(^{2+}\)] rise started within a few seconds after the beginning of the ACPD application, reached a peak in 10.8 ± 1.5 s, and then recovered to baseline in 59.4 ± 4.0 s (Fig. 5A, top trace; see also Fig. 5B, top graph). The half-amplitude duration of the transient [Ca\(^{2+}\)] rise was 26.8 ± 2.6 s.

**Pharmacology of the ACPD-induced responses**

MCPG, an antagonist effective against both group I and group II mGluR-mediated responses, prevented the development of the ACPD-induced depolarization and the intracellular Ca\(^{2+}\) responses when added to the perfusing solution (0.5–1 mM; \(n = 4\); Fig. 6). 4C3HPG (0.5 mM), an agonist of group II mGluRs and an antagonist of the group I mGluRs, also prevented the development of the ACPD-induced intracellular Ca\(^{2+}\) and voltage responses (\(n = 3\)). These results indicate that the responses activated by ACPD are mediated by activation of group I mGluRs. We used the selective group I mGluR agonist DHPG (50 \(\mu\)M; \(n = 6\)) to confirm the involvement of these receptors. DHPG induced depolarizations and biphasic [Ca\(^{2+}\)]\(_i\) rises. When the responses were recorded at hyperpolarized levels, DHPG elicited a transient [Ca\(^{2+}\)]\(_i\) rise similar to that elicited by ACPD (Fig. 7).

**Effect of caffeine on the voltage and [Ca\(^{2+}\)]\(_i\) responses induced by ACPD**

One possible source for the voltage-independent transient [Ca\(^{2+}\)]\(_i\) increase is release from intracellular stores. Caffeine has been reported to affect this process in a reversible way (Friel and Tsien 1992; Henzi and MacDermott 1992). We tested the effect of caffeine on the ACPD-induced voltage and Ca\(^{2+}\) responses in CA3 pyramidal cells. ACPD was applied in the absence, in the presence, and after wash out of caffeine (bath-applied 2–5 mM; Fig. 8). In the presence of caffeine, ACPD induced the expected depolarization and sustained [Ca\(^{2+}\)]\(_i\) rise, but the initial transient [Ca\(^{2+}\)]\(_i\) response was blocked [Fig. 8A, Caffeine (bath); \(n = 5\)]. The transient [Ca\(^{2+}\)]\(_i\) response reappeared after wash out of caffeine (Fig. 8A, Wash). These observations were confirmed in four cells (Fig. 8B). Thus the transient [Ca\(^{2+}\)]\(_i\) rise was not required for the ACPD-induced depolarization. Bath application of caffeine alone sometimes caused a small depolarization (2–6 mV; \(n = 3\) of 5). However, when the holding current was adjusted to keep the membrane potential constant, caffeine did not cause steady-state changes in [Ca\(^{2+}\)]\(_i\). We next confirmed that, as previously reported (Garaschuk et al. 1997; Glaum et al. 1990), pulse application of caffeine caused transient Ca\(^{2+}\) release. Figure 8C shows that pulse application of caffeine elicited a transient [Ca\(^{2+}\)]\(_i\) rise similar to the transient [Ca\(^{2+}\)]\(_i\) rise elicited by ACPD (\(n = 10\)).

**Input resistance change underlying the ACPD-induced depolarization**

Input resistances (\(R_{en}\)) of CA3 pyramidal neurons were assessed by current injections. Ramp current injections were applied over ranges of −80 to −45 mV before and after ACPD application (Fig. 9). Figure 9B shows the average voltage-current relationship (V-I plot) from five experiments. The V-I plot suggests that in control conditions a decrease in membrane resistance occurred at membrane potentials more depolarized than −60 mV (inward rectification). This rectification was suppressed by ACPD. ACPD also increased the input resistance of the cells from 67.0 ± 5.9 MΩ to 96.3 ± 8.4 MΩ (\(P < 0.001\); \(n = 5\); Student’s t-test for paired data). The extrapolated intersection of the regression lines for the control and the ACPD responses was at −102.9 ± 4.0 mV, near the potassium equilibrium potential. Simultaneous monitoring of the Ca\(^{2+}\) response suggests that the threshold for voltage-gated Ca\(^{2+}\) entry was not altered by ACPD (\(n = 4\); \(P = 0.36\); Student’s t-test for paired data; Fig. 9). The V-I plot obtained during the ACPD response also showed no significant change in

**FIG. 7.** Comparison of intracellular voltage and Ca\(^{2+}\) responses elicited by ACPD and (S)-3,5-dihydroxyphenylglycine (DHPG). Applications of ACPD (A) and of the selective group I mGluR agonist DHPG (B) to the same cell. Resting membrane potentials: A, −70 mV; B, −73 mV. Both agonists elicited a voltage-independent, transient [Ca\(^{2+}\)]\(_i\) rise (arrow). In this cell, the threshold for voltage-dependent Ca\(^{2+}\) entry was estimated before application of agonists and was −58 mV (A) and −59 mV (B).
Intracellular voltage and calcium responses to mGluR agonists in CA1 cells

Our data indicate that the mGluR-mediated depolarization in CA3 neurons was not caused by an increase of [Ca$^{2+}$], because neither sustained (Fig. 4D) nor transient (Fig. 8) [Ca$^{2+}$]$_i$ rise was required for the depolarization. To the contrary, studies in CA1 cells have shown that a depolarizing response to mGluR stimulation is sustained by a calcium-activated nonspecific cation current ($I_{\text{CAN}}$) (Congar et al. 1997; Crépel et al. 1994). We carried out additional experiments in CA1 cells to evaluate whether the mGluR responses we recorded in CA3 could also be found in CA1 and whether the sustained depolarization in CA1 required a [Ca$^{2+}$]$_i$ rise.

In all the CA1 cells examined at resting potential ($n = 13$), ACPD elicited [Ca$^{2+}$]$_i$ increases and membrane potential changes. As in CA3 cells, the [Ca$^{2+}$]$_i$ response consisted of a transient rise followed by a sustained elevation. The sustained phase of the [Ca$^{2+}$]$_i$ response was suppressed by hyperpolarization (Fig. 10). The voltage response consisted of an initial hyperpolarization followed by a long-lasting depolarization (Fig. 10). DHPG elicited intracellular Ca$^{2+}$ and voltage responses similar to the ACPD-induced ones ($n = 4$; not shown).

The initial hyperpolarization elicited by mGluR agonists was observed in all CA1 cells ($n = 16$; Figs. 10 and 11), whereas
FIG. 9. Voltage-current (V-I) relationship before and after ACPD application. A: 2 depolarizing ramps (I) were applied to a CA3 pyramidal cell; the 1st one before the ACPD application; the 2nd ramp during the ACPD-induced depolarization. The resting membrane potential was −72 mV before the 1st depolarizing ramp and before the ACPD application. Hyperpolarizing current was required to reset the membrane potential to −72 mV before the 2nd depolarizing ramp because of the ACPD-induced depolarization. Rm before and during the ACPD response (just before the ramps, both at −72 mV) was 70 and 104 MΩ, respectively. The Ca²⁺ trace (Ca²⁺) was corrected for bleaching that was 6% of the baseline fluorescence (see METHODS). B: summary data, n = 4 cells. V-I plots obtained from responses to the depolarizing ramp before (Control; ○) and after (ACPDS; ●) the application of ACPD. Each symbol is an average of 2–5 values; error bars, SE. Plots were fitted with linear regressions over current ranges of 0.200–0.100 nA for Control (R² = 0.995) and of −0.325–0.050 nA for ACPD (R² = 0.996). Note the rectification of the control curve at depolarized voltages and the increased slope of the ACPD curve. Arrows indicate the voltage thresholds for Ca²⁺ rise (mean ± SE; n = 4) estimated from the Ca²⁺ responses during the depolarizing ramps in control (−) and in the presence of ACPD (→). The slope of the ACPD curve is not appreciably different at voltages above the threshold for Ca²⁺ rise.

It was only occasionally seen in CA3 cells (10 of 67; e.g., Fig. 8A). A comparison of the averaged transient intracellular Ca²⁺ response recorded in CA1 cells with that of the averaged hyperpolarizing voltage response shows that the two events peaked at approximately the same time (Fig. 11).

The effect of membrane potential on the mGlur-mediated responses in CA1 cells was examined. ACPD was applied at different initial membrane potentials (Fig. 12B). However, CA1 cells were more difficult to hyperpolarize than CA3 cells, presumably because hyperpolarization activated the “Q” current (Fig. 12A, left), which acted to shunt the injected current. For this reason, some experiments were performed using QX-314 in the recording pipette to block the Q current (Perkins and Wong 1995) (Fig. 12A, right). In Fig. 12B, increasing levels of hyperpolarization gradually suppressed the sustained Ca²⁺ response. With sufficient hyperpolarization, the sustained Ca²⁺ component was completely blocked, leaving behind an isolated transient [Ca²⁺]i elevation. As in CA3 pyramidal cells, the sustained Ca²⁺ component was suppressed by membrane hyperpolarization.

FIG. 10. Responses of a CA1 pyramidal cell to ACPD application and to current injection. As in Fig. 9, a 1st depolarizing ramp (I) was applied before and a 2nd one after the ACPD application. The resting membrane potential was −55 mV (−, Vm). The depolarizing ramps were applied from a membrane potential of −64 mV. ACPD induced a sustained depolarization that was accompanied by a transient [Ca²⁺]i rise (Ca²⁺, arrow) followed by a sustained [Ca²⁺]i elevation. In CA1 cells, the sustained Ca²⁺ component was suppressed by membrane hyperpolarization.

FIG. 11. Effects of ACPD on CA1 pyramidal neurons. A: in a CA1 pyramidal cell the ACPD-induced depolarization was preceded by a transient hyperpolarization (Vm). As in CA3 neurons, ACPD induced 2 phases of [Ca²⁺]i increase (Ca²⁺), an initial transient [Ca²⁺]i rise (arrow) followed by a sustained component of elevated [Ca²⁺]i. Both the voltage and Ca²⁺ responses recovered to baseline values by −15 min after the ACPD application (not shown). Initial membrane potential −56 mV. B: fluorescence (○) and voltage changes (●) after ACPD application were averaged for 5 applications in 3 cells. Traces were sampled at 1 Hz, and the different applications were aligned at the onset of the calcium responses. Error bars, SE. The average membrane potential before ACPD application was −61.6 ± 2.9 mV. The transient [Ca²⁺]i rise (arrow) was consistently associated with the transient hyperpolarization.
unlikely to be influx through voltage-gated Ca\(^{2+}\) channels. A more likely source is release from intracellular stores. The reversible blockade of the transient [Ca\(^{2+}\)]\(i\) rise by caffeine also suggests an involvement of intracellular Ca\(^{2+}\) stores. Activation of group I mGluRs has been shown to generate IP\(_3\) and to release Ca\(^{2+}\) from IP\(_3\)-sensitive stores (Abe et al. 1992; Masu et al. 1991; Pozzo Miller et al. 1996). Although caffeine and IP\(_3\) may act on different intracellular Ca\(^{2+}\)-permeable channels, co-localization of these channels in hippocampal cells has been demonstrated (Seymour-Laurent and Barish 1995; Sharp et al. 1993), suggesting the possibility that caffeine and IP\(_3\) act on the same intracellular Ca\(^{2+}\) store. We showed that pulse application of caffeine caused transient [Ca\(^{2+}\)]\(i\) increase (Fig. 8C). Bath application of caffeine may deplete a common pool of releasable Ca\(^{2+}\) (Seymour-Laurent and Barish 1995) and thereby prevent the occurrence of the mGluR-mediated [Ca\(^{2+}\)]\(i\) transient. Alternatively, a direct antagonistic action of caffeine on IP\(_3\) receptors (Ehrlich et al. 1994; Parker and Ivorra 1991; Seymour-Laurent and Barish 1995) may be involved.

The amplitude of the ACPD-induced transient [Ca\(^{2+}\)]\(i\) rise was small compared with that elicited by a burst of action potentials. In CA1 cells, however, it was probably sufficient to activate a calcium-dependent K\(^+\) current (see below). Another possible role for the transient [Ca\(^{2+}\)]\(i\) rise would involve initiation of [Ca\(^{2+}\)]\(i\) waves (Berridge 1997). Agonist-induced release of Ca\(^{2+}\) from intracellular stores can set up propagating [Ca\(^{2+}\)]\(i\) waves (Jaffe and Brown 1994). Propagating [Ca\(^{2+}\)]\(i\) increases may provide the link between receptor responses and protein synthesis (Berridge 1998; Phenna et al. 1995). Other studies from this laboratory have shown that group I mGluR activation elicits protein synthesis-dependent long-term changes in the activity of hippocampal CA3 cells (Merlin et al. 1998). It would be interesting to test whether the transient [Ca\(^{2+}\)]\(i\) rise we have observed plays a role in this phenomenon.

Sustained [Ca\(^{2+}\)]\(i\) rise is mediated by influx through voltage-gated Ca\(^{2+}\) channels

The contribution of voltage-dependent Ca\(^{2+}\) channels to the sustained [Ca\(^{2+}\)]\(i\) rise is supported by the observation that the accumulation of [Ca\(^{2+}\)]\(i\) during the sustained component occurred only when the neuron was depolarized above the threshold for voltage-dependent Ca\(^{2+}\) influx (see Fig. 3B). Hyperpolarization during the sustained calcium rise to levels below the threshold also reversibly and totally suppressed the Ca\(^{2+}\) accumulation (see Fig. 9A). The average threshold for voltage-dependent [Ca\(^{2+}\)]\(i\) increase was about −60 mV. Although low-threshold T-type Ca\(^{2+}\) channels are present in hippocampal pyramidal cells (Magee et al. 1995; Soong et al. 1993), one might expect these channels to be inactivated at the potentials at which our recordings were carried out (Kavalali et al. 1997). Sustained low-voltage-activated Ca\(^{2+}\) currents have also been described in both CA1 (Magee et al. 1996) and CA3 (Avery and Johnston 1996) pyramidal cells; such currents are a more likely source of the Ca\(^{2+}\) in the group I mGluR-mediated sustained calcium response. Pharmacological characterization of these currents was not attempted in this study, because blockers of voltage-gated Ca\(^{2+}\) channels were reported to affect the function of intracellular Ca\(^{2+}\) stores, presumably by preventing refilling of depleted stores (Garaschuk et al. 1997).
Mechanisms of the mGluR-mediated membrane potential changes

In CA1 cells, ACPD elicited an initial hyperpolarization that was associated with the transient [Ca\(^{2+}\)] increase. Hyperpolarizing responses elicited by ACPD have been observed previously in CA1 cells and were attributed to the activation of Ca\(^{2+}\)-dependent K\(^+\) conductances (Jaffe and Brown 1994; Shirasaki et al. 1994). QX-314 has been shown to suppress a component of the Ca\(^{2+}\)-dependent K\(^+\) current (Oda et al. 1992). Thus the block of the hyperpolarization by QX-314 (Fig. 12) is consistent with the notion that this response was caused by a Ca\(^{2+}\)-dependent K\(^+\) current triggered by the transient [Ca\(^{2+}\)] rise. The mGluR-mediated hyperpolarizing responses were more consistently expressed in CA1 cells than in CA3 cells. Because CA3 pyramidal cells are known to exhibit a Ca\(^{2+}\)-dependent K\(^+\) current (Schwartzkroin and Stafstrom 1980; Storm 1990), our observation suggests that the mGluR-mediated [Ca\(^{2+}\)]\(_i\), rise in CA1 cells can more easily access membrane K\(^+\) channels than can the [Ca\(^{2+}\)]\(_i\), rise in CA3 cells.

Both CA1 and CA3 cells exhibited a sustained depolarization in response to ACPD. It has been suggested that an mGluR-mediated depolarization in CA1 pyramidal cells is due to a nonspecific cation current activated by Ca\(^{2+}\) release from intracellular stores (Congar et al. 1997; Crépel et al. 1994). We found an mGluR-mediated depolarization in both CA1 and CA3 cells that far outlasted the transient Ca\(^{2+}\) elevation due to intracellular release (Figs. 12B and 4). Moreover, the depolarization persisted in the absence either of the transient (Fig. 8, A and B) or of the sustained (Figs. 5 and 12) components of increased [Ca\(^{2+}\)], making it unlikely to be the result of the Ca\(^{2+}\)-activated current described previously.

The ACPD-induced depolarization led to a voltage-dependent Ca\(^{2+}\) influx. However, the ACPD V-I plot (Fig. 9) suggests that the voltage-dependent Ca\(^{2+}\) currents did not contribute significantly to the depolarization. If the depolarization caused by the Ca\(^{2+}\) current were large, one would expect a departure of the slope of the V-I curve from linearity.

mGluR-mediated depolarizations have been reported in both CA1 and CA3 that result from conductance increases (Congar et al. 1997; Crépel et al. 1994; Guérineau et al. 1995; Pozzo Miller et al. 1995). On the other hand, the long depolarization that we recorded was associated with a conductance decrease (i.e., increase in input resistance). mGluR-mediated depolarizations involving conductance decreases have also been reported by others and attributed to blockade of K\(^+\) channels (Charpak et al. 1990; Guérineau et al. 1994; Lüthi et al. 1997).

Appropriately, the mGluR-mediated depolarization in hippocampal pyramidal cells consists of multiple components. Differences in recording conditions may affect the relative expression of the different components. For instance, the depolarizing conductance increases were recorded in the presence of potassium channel blockers (Congar et al. 1997; Crépel et al. 1994) or elevated extracellular [K\(^+\)] (Guérineau et al. 1995), whereas under normal ionic conditions, a depolarization associated with a conductance decrease was more prominent (Guérineau et al. 1994; and this study). It is notable that mGluR-mediated depolarizations are sufficient to activate voltage-dependent Ca\(^{2+}\) influx and thus to modulate [Ca\(^{2+}\)]\(_i\), even near the resting membrane potential.

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