1S,3R-ACPD Induces a Region of Negative Slope Conductance in the Steady-State Current-Voltage Relationship of Hippocampal Pyramidal Cells

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INTRODUCTION

The suppression of K⁺ conductances is a major effect associated with the activation of postsynaptic metabotropic glutamate receptors (mGluRs) and muscarinic receptors in hippocampal pyramidal cells. At least four K⁺ currents, after-hyperpolarizing (I_{AHP}), I_{M}, I_{leak}, and I_{slow}, are decreased with the stimulation of these G protein-coupled receptors, leading to an enhancement in cellular excitability (Brown and Adams 1980; Charpak et al. 1990; Gerber and Gähwiler 1994; Lüthi et al. 1996). Under voltage-clamp conditions, this effect is in part reflected by a slowly developing inward current associated with a decrease in input conductance. In CA3 cells, the response is observed both with exogenous application of agonists as well as with synaptic stimulation of the receptors (Charpak and Gähwiler 1991; Gähwiler and Brown 1985; Gerber and Gähwiler 1994; Gerber et al. 1993).

Synaptic responses mediated by mGluRs and metabotropic cholinergic receptors are highly voltage dependent, disappearing at potentials below −70 mV while disproportionately increasing positive to resting potential (Charpak and Gähwiler 1991; Gähwiler and Brown 1985; Wang and McCormick 1993). For mGluRs, an increase in amplitude of more than fourfold is observed when responses are evoked at a potential of −54 mV compared with resting potential (about −64 mV) (Charpak and Gähwiler 1991). The increase in driving force for K⁺ ions and the partial activation of I_{M} (Brown 1988; Gähwiler and Brown 1985) cannot quantitatively account for this voltage dependence. Activation of mGluRs at depolarized potentials may therefore suppress additional K⁺ currents or induce depolarizing inward currents. Alternatively, intrinsic voltage-dependent nonlinearities in the mGluR-mediated response may contribute to the increase of the slow inward current on depolarization. For example, the gating of K⁺ channels by an inhibitory G protein or second messenger may be sensitive to changes in membrane potential.

Activation of G protein-coupled receptors often results in more pronounced effects on membrane currents with more moderate than with strong depolarization, as demonstrated for the inhibition of N-type Ca²⁺ channels by norepinephrine, glutamate (acting at mGluRs), γ-amino-butyrate, luteinizing-hormone-releasing hormone, and kappa opioid receptor agonists (Anwyl 1991; Bean 1989; Boland and Bean 1993; Dolphin 1996; Luebke and Dunlap 1994; Swartz and Bean 1992). This is thought to result from a G protein-induced conversion of the channels into a gating mode that is less "willing" to open on depolarization (Bean 1989; Boland and Bean 1993; Diversé-Pierluissi et al. 1995). For the gating of K⁺ conductance by mGluRs, the nature of the transduction process is unclear and the characterization of a voltage dependence should provide insights concerning the mechanisms coupling receptors to channels.
The aims of this study were to electrophysiologically characterize the voltage dependence of the metabotropic glutamatergic response in hippocampal pyramidal cells and to evaluate the physiological implications. Preliminary results have appeared in abstract form (Lüthi et al. 1995).

METHODS

Hippocampal slice cultures

Slices 400 μm thick were obtained from 6-day-old rat pups and cultured by means of the roller tube technique for 3–6 wk as described previously (Gähwiler 1981).

Electrophysiological recordings

The single-electrode voltage clamp was used to record from CA3 pyramidal cells (Axoclamp2 amplifier, Axon Instruments, Foster City, CA). For this purpose, cultures were transferred to a temperature-controlled recording chamber that was mounted on the stage of an inverted microscope (Axiovert 35M, Zeiss, Jena, Germany) and superfused with a saline solution (33°C) containing (in mM) 149.2 Na⁺, 2.7 K⁺, 147.2 Cl⁻, 2.8 Ca²⁺, 0.5 Mg²⁺, 11.6 HCO₃⁻, 0.4 H₂PO₄⁻, 5.6 D-glucose, and 0.0005 tetrodotoxin. For experiments with high K⁺ (8 mM), NaCl content was reduced correspondingly. When Ba²⁺-containing solutions (1 mM) were used, H₂PO₄⁻ was omitted from the saline to avoid precipitation of phosphate salts and pH was readjusted. Low-Ca²⁺ saline contained 0.5 mM Ca²⁺ and 10 mM Mg²⁺ instead of the concentrations given above. Solutions were brought to a pH of 7.4 by bubbling with 95% O₂-5% CO₂ and monitored with Phenol Red (10 mg/l).

For microelectrode recordings, thin-walled electrodes were filled with a solution containing (in M) 2 KCl, 2 CsCl, or 1 KMeSO₄, resulting in a tip resistance of 30–50 MΩ for KCl and CsCl and 60–80 MΩ for KMeSO₄, respectively. During voltage-clamp recordings, the switching rate ranged between 1.7 and 2.0 kHz and headstage output was continuously monitored to ensure adequate settling time between samples. After voltage steps, the clamp stabilized within 5–10 ms. Input resistance was assessed by applying 0.5-s hyperpolarizing voltage commands of 10 mV.

Microelectrode signals were digitized at 1–2 kHz, stored, and analyzed off-line on a PC with the use of pClamp version 6.0.1 (Axon Instruments, Foster City, CA) and Fig.P (Biosoft, Cambridge, UK) software. Quantitative data are given as means ± SE. Statistical analysis was performed with the use of Student’s t-test.

Drugs and chemicals

Drugs were applied in the bathing fluid and were purchased from the following sources: tetraethylammonium chloride (TEA) and muscarine from Sigma (St. Louis, MO); 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD) and N-methyl-d-aspartate (NMDA) from Tocris Neuramin (Bristol, UK); bis-(α-amino-phenoxyl)-N,N,N’,N’-tetraacetic acid (BAPTA) from Molecular Probes (Eugene, OR); and tetrodotoxin from Sankyo (Tokyo, Japan).

RESULTS

Negative slope conductance in the steady-state current-voltage relationship in cells bathed in 1S,3R-ACPD

The effect of mGluR activation on the steady-state I-V relationship of CA3 pyramidal neurons was determined by bath applying 1S,3R-ACPD (10 μM), a specific agonist for mGluRs. At this concentration, 1S,3R-ACPD effectively suppresses K⁺ currents in pyramidal neurons of organotypic slice cultures (Lüthi et al. 1994). Under control conditions, increasing the amplitude of hyperpolarizing steps in 10-mV increments to −90 mV evoked passive current responses (Fig. 1). In contrast, depolarizing pulses resulted in rapid activation of transient currents followed by slowly developing outward currents that reached a maximal amplitude after 200–600 ms, as described previously (Brown and Griffith 1983; Brown et al. 1990; Segal and Barker 1984).

In this study, we have focused on the characterization of metabotropic actions on the steady-state components of the depolarization-induced responses. Bath application of 1S,3R-ACPD (10 μM) for 1–2 min at −60 mV induced an inward current of −181 ± 35 pA (n = 19), as demonstrated earlier (Charpak and Gähwiler 1991; Charpak et al. 1990; Gerber et al. 1993). In marked contrast to the control outward currents evoked by positive voltage steps, identical depolarizing voltage commands from −60 mV in the presence of 1S,3R-ACPD induced additional inward current despite the already fully developed mGluR response. Hyperpolarizing steps in the presence of 1S,3R-ACPD evoked passive current responses of decreased amplitude compared with control (44.8 ± 7.1% of control for 10-mV hyperpolarizing steps, n = 12; 72.3 ± 6.9% of control for 30-mV hyperpolarizing...
steps, n = 12) due to the suppression of resting K\(^+\) conductances (Gerber and Gähwiler 1994). As a result, the outwardly rectifying steady-state I-V relationship of CA3 neurons under control conditions was converted to an S-shaped curve exhibiting a region of negative slope conductance between −60 and −40 mV in the presence of 1S,3R-ACPD (Fig. 1B). Similar findings were obtained when muscarinic cholineric receptors were activated. In the continuous presence of muscarine (10 μM), a 20-mV depolarization from resting potential evoked an inward current with an amplitude peaking at −40 mV (Fig. 2, A–C, n = 5). Under these conditions of muscarinic receptor activation, application of 1S,3R-ACPD (10 μM) induced no significant additional response (Fig. 2C, n = 3).

The nonlinearity of the 1S,3R-ACPD-response was not dependent on whether the step depolarization preceded drug application, or vice versa. Thus the absolute holding current level reached with a depolarization to −50 mV in the continuous presence of 1S,3R-ACPD was not significantly different from that attained when 1S,3R-ACPD was applied to a cell already held at −50 mV (−283 ± 30 pA vs. −275 ± 29 pA, n = 3, P > 0.05, not shown).

**Kinetics of the 1S,3R-ACPD-dependent current induced on depolarization**

The development of the depolarization-induced inward current in the continuous presence of 1S,3R-ACPD was kinetically best described by a single-exponential fit giving a time constant of 800 ± 262 ms (at −50 mV, n = 10). At −40 mV, the current peaked earlier, with a time constant of 281 ± 49 ms (n = 5). The 1S,3R-ACPD-sensitive inward current at −50 or −40 mV tended to decrease when the depolarization was maintained for >5 s, suggesting desensitization of the metabotropic response.

**Depolarization-induced 1S,3R-ACPD current is due to a suppression of K\(^+\) current**

The negative slope region in the I-V relationship in the presence of 1S,3R-ACPD could be explained by 1) a decrease in 1S,3R-ACPD-sensitive K\(^+\) currents or 2) an induction of an inward current.

The reversal potential of the depolarization-evoked current in the presence of 1S,3R-ACPD was determined by measuring the amplitude of the 1S,3R-ACPD-sensitive instantaneous currents as a function of the holding potential. Tail currents were evoked with the use of a two-pulse procedure (Hodgkin and Huxley 1952). Cells were voltage clamped at −40 mV for 1 s to activate the 1S,3R-ACPD-sensitive current, and the membrane potential was then stepped to between −90 and −50 mV for 1 s. The family of currents generated in this way is shown in Fig. 3. The 1S,3R-ACPD-sensitive tail currents (Fig. 3C) were isolated by digitally subtracting the control tail currents (Fig. 3A) from the tail currents in the presence of 1S,3R-ACPD (Fig. 3B). The amplitude of these responses, measured 20 ms after the voltage step, was plotted against the clamp potential, resulting in a linear relationship (Fig. 3E, ●). The fitted curve intersects the zero-current line at −93.0 ± 2.2 mV (n = 6), close to the reversal potential for K\(^+\) ions in CA3 cells, as previously determined (about −100 mV, Lüthi et al. 1996). When extracellular K\(^+\) was raised from 2.7 to 8 mM, the reversal potential for the current was shifted positively to −76.9 ± 5.1 mV (n = 3; Fig. 3, D and E). This is in reasonable agreement with the predicted potential of −72 mV according to the Nernst equation.

Three additional series of experiments supported the conclusion that the 1S,3R-ACPD-sensitive current is carried by K\(^+\) ions. First, in cells internally perfused with 2 M CsCl, 1S,3R-ACPD-dependent current was not detected at depolarized potentials (n = 6, not shown). Second, in the presence of 1 mM extracellular Ba\(^{2+}\) (in low-Ca\(^{2+}\) saline), the effect of 1S,3R-ACPD on the I-V relationship was markedly reduced (Fig. 4). Third, when electrodes were filled with 2 M KMeSO\(_4\) instead of 2 M KCl to change the reversal potential for Cl\(^-\) in the hyperpolarizing direction (Streit et al. 1989), the action of 1S,3R-ACPD remained unaltered, indicating that a Cl\(^-\) conductance was not involved (n = 5, not shown).

**FIG. 2.** Depolarization induces an inward current in the continuous presence of muscarine. Top 2 traces: membrane currents. Bottom: membrane potential. Current responses to a step depolarization from −60 to −40 mV and to a step hyperpolarization from −60 to −80 mV are shown. A: under control conditions, depolarization induces a delayed outward current and hyperpolarization induces a passive current response. B: in the presence of muscarine, depolarization evokes an inward current. Hyperpolarizing responses are largely passive but occasionally show a small outward current. C: pooled data showing the steady-state I-V relationship in the presence and absence of muscarine (n = 5), and the occlusion of the 1S,3R-ACPD response by muscarine (n = 3).
steady-state $I-V$ relationship did not show a region of negative slope conductance and outward rectification was largely maintained. A similar result was obtained when increases in intracellular Ca$^{2+}$ concentration were prevented with the Ca$^{2+}$ chelator BAPTA (100 mM in the pipette, $n = 6$, not shown). Moreover, in the presence of TEA (10 mM), an effective blocker of $I_M$ in hippocampal pyramidal cells (Storm 1989), depolarization did not induce inward currents, even though TEA was effective in reducing delayed outward currents. Furthermore, TEA did not occlude the depolarization-induced inward currents.

Inhibition of $I_{AHP}$ and $I_M$ does not induce a region of negative slope conductance

Experiments were performed to check whether the nonlinearity of the 1S,3R-ACPD response apparent at depolarized potentials was due to suppression of the K$^+$ currents $I_{AHP}$ and $I_M$. The effects of 1S,3R-ACPD were therefore compared with those of K$^+$ channel blockers (Fig. 5). First, cells were bathed in a solution containing low-Ca$^{2+}$ (0.5 mM) and high-Mg$^{2+}$ saline (10 mM) to prevent activation of Ca$^{2+}$-dependent K$^+$ currents. Although this solution was effective in blocking the $I_{AHP}$ evoked by a 50- to 2,000-ms depolarizing voltage command of 30–40 mV (not shown), the resulting $I-V$ relationship did not show a region of negative slope conductance and outward rectification was largely maintained. A similar result was obtained when increases in intracellular Ca$^{2+}$ concentration were prevented with the Ca$^{2+}$ chelator BAPTA (100 mM in the pipette, $n = 6$, not shown). Moreover, in the presence of TEA (10 mM), an effective blocker of $I_M$ in hippocampal pyramidal cells (Storm 1989), depolarization did not induce inward currents, even though TEA was effective in reducing delayed outward currents. Furthermore, TEA did not occlude the depolarization-induced inward currents.

![Fig. 4](image-url)  

**Fig. 4.** Ba$^{2+}$ (1 mM, 0.5 mM Ca$^{2+}$, 10 mM Mg$^{2+}$) reduces the effects of 1S,3R-ACPD. A: representative current responses in a cell clamped at $-60$ mV to voltage commands from $-90$ to $-40$ mV for 2 s. B: plot of current amplitudes in the absence (○) and presence (●) of 1S,3R-ACPD.

![Fig. 5](image-url)  

**Fig. 5.** Effect of 1S,3R-ACPD (●) is not mimicked by K$^+$ channel blocking solutions. A: low-Ca$^{2+}$ solution (0.5 mM Ca$^{2+}$, 10 mM Mg$^{2+}$) (△) and tetraethylammonium chloride (TEA) (10 mM) in low-Ca$^{2+}$ solution (○) were tested. B: application of these solutions reduced delayed outward currents, but did not result in a negative slope conductance in the $I-V$ relationships. The curve for 1S,3R-ACPD is shown for comparison.
Depolarization-evoked 1S,3R-ACPD-sensitive current is Ca\(^{2+}\) independent

Experiments were performed in the presence of low-extra-
cellular-Ca\(^{2+}\) saline (0.5 mM Ca\(^{2+}\), 10 mM Mg\(^{2+}\)) to test for a Ca\(^{2+}\) dependence of the 1S,3R-ACPD-dependent current. Mg\(^{2+}\) itself was, however, found to reduce the amplitude of the 1S,3R-ACPD-induced inward current observed at resting potentials [80 ± 18 pA (n = 5) vs. 208 ± 25 pA (n = 8), P < 0.0025] and concomitantly diminished the effects of 1S,3R-ACPD on depolarization. The effects of Ca\(^{2+}\) at 2.8 and 0.5 mM were therefore compared (in the presence of 10 mM Mg\(^{2+}\)). In both cases, the depolarization-induced inward current was of comparable amplitude (n = 8, P > 0.05, not shown), indicating that the reduction of extracellular Ca\(^{2+}\) did not affect this action of 1S,3R-ACPD. In further support for the Ca\(^{2+}\) independence of the 1S,3R-ACPD-sensitive current, intracellular perfusion of cells with high concentrations of the Ca\(^{2+}\) buffer BAPTA (100 mM) did not prevent this effect of 1S,3R-ACPD (n = 6, not shown). To check for an involvement of an inward rectifier current (Hal-liswell and Adams 1982) 1S,3R-ACPD was applied in the presence of 1 mM extracellular Cs\(^{+}\). The amplitudes of the inward currents evoked with 10- to 20-mV depolarizing steps did not significantly differ from the amplitudes of the currents induced by 1S,3R-ACPD alone (−26 ± 41 pA vs. −35 ± 24 pA at −50 mV; −176 ± 71 pA vs. −247 ± 53 pA at −40 mV, n = 6, P > 0.05). This suggests that Cs\(^{+}\)-sensitive inward rectifiers do not contribute to the current induced on depolarization in the presence of 1S,3R-ACPD.

Comparison of the effects of 1S,3R-ACPD and NMDA

A region of negative slope conductance in the I-V relationship is a prominent feature associated with the activation of NMDA receptors in the presence of Mg\(^{2+}\) ions (Mayer et al. 1984; Nowak et al. 1984). The parallels observed in this study with regards to the effects of 1S,3R-ACPD on the I-V relationship prompted us to compare the actions of agonists for these two glutamate receptor types as a function of the Mg\(^{2+}\) concentration (Fig. 6). When NMDA was applied in the presence of Mg\(^{2+}\) (2 mM), a 10-mV depolarization rapidly induced an inward current, as expected for immediate depolarization-induced relief of the Mg\(^{2+}\) blockade in the receptor pore (Fig. 6B). This rectification was, however, absent when extracellular Mg\(^{2+}\) was removed from the perfusate. In this case, NMDA-receptor-mediated currents were observed as linear currents summatting with the cellular response (Fig. 6C). In contrast, the 1S,3R-ACPD-sensitive current appeared not to be affected by Mg\(^{2+}\) in the concentration range between 0 and 2 mM (Fig. 6, B and C). Repetitive depolarizations from −60 to −50 mV evoked a delayed inward current, showing that the 1S,3R-ACPD response recovered fully during the course of the decay of the outward tail current.

DISCUSSION

This study demonstrates that the reduction of K\(^{+}\) conductance in response to mGluR and muscarinic receptor activation exhibits pronounced voltage sensitivity. After full development of the mGluR-mediated suppression of K\(^{+}\) currents at resting potential, moderate depolarization induced an additional inward current with a slow onset. This effect is apparent as a region of negative slope conductance in the steady-state I-V relationship of hippocampal pyramidal cells.

The negative slope conductance in the I-V relationship with 1S,3R-ACPD is likely to correspond to the augmentation of evoked synaptic currents observed with depolarization from the resting potential (Charpak and Gähwiler 1991). The holding current levels reached at the peak of the responses were independent of the sequence of depolarization and drug application, indicating that the depolarization-induced enhancement of mGluR responses is independent of the recent voltage history of the cell. Therefore both the depolarization-evoked 1S,3R-ACPD-dependent inward current and the enhancement of synaptic responses on depolarization appear to reflect a slowly developing action on a steady-state membrane current.

Identity of the underlying current

The 1S,3R-ACPD-sensitive tail currents reversed close to the equilibrium potential for K\(^{+}\) ions. With low extracellular [K\(^{+}\)] the reversal potential was determined by extrapolation, because it was not possible to experimentally reverse the polarity of the current responses. This may reflect the weakened action of 1S,3R-ACPD at hyperpolarized membrane potentials. When extracellular K\(^{+}\) was increased, however, a reversal in the polarity of the tail currents was observed. Furthermore, the 1S,3R-ACPD-induced current was significantly reduced in the presence of the K\(^{+}\)-channel-specific blockers, internal Cs\(^{+}\) or external Ba\(^{2+}\).

The negative slope conductance could also be due, in part, to activation of 1S,3R-ACPD-gated inward currents. It has previously been shown that 1S,3R-ACPD can activate cationic currents (Caeser et al. 1993; Crépel et al. 1994; Guéneau et al. 1995). In these studies, however, a cationic current response was only observed with strong depolarizing steps positive to −30 mV, with high concentrations of 1S,3R-ACPD (100 μM) or under conditions of high external K\(^{+}\).

Thus in our experiments the contribution from cationic currents should be minor.

Possible mechanisms: voltage-dependent K\(^{+}\) current versus intrinsic voltage sensitivity of 1S,3R-ACPD actions

The action of 1S,3R-ACPD could not be mimicked by applying K\(^{+}\) channel blocking solutions that reduce the 1S,3R-ACPD-sensitive currents \(I_{\text{M}}\) and \(I_{\text{AMP}}\). This suggests that either the reduction of an unknown voltage-dependent K\(^{+}\) current or an intrinsic voltage-dependent enhancement of the action of 1S,3R-ACPD on K\(^{+}\) currents accounts for the region of negative slope conductance.

On the basis of the activation kinetics and the sustained action of the 1S,3R-ACPD effect, a putative current fulfilling the properties required to produce the voltage-dependent response would have to be a member of the slowly or noninac-
activating class of K⁺ currents, such as Iₖ (Brown et al. 1990; Storm 1993). The experimental results with high concentrations of TEA suggest that reduction of Iₖ is not the primary cause for the voltage-dependent action of 1S,3R-ACPD, although we cannot rule out a partial contribution by Iₖ. Moreover, the involvement of a novel voltage-dependent tonic current with an activation threshold near −55 mV current appears unlikely. The current would have to attain a sufficient amplitude with moderate depolarizations for its suppression to fully occlude the sum of all the other depolarization-activated and tonic K⁺ currents.

The alternative possibility is an intrinsic voltage sensitivity in the transduction mechanism mediating the suppression of a voltage-independent K⁺ conductance. Such a process has been proposed to explain the voltage-dependent actions of 1S,3R-ACPD and muscarinic agonists in cortical layer V cells (Wang and McCormick 1993). Activation of mGluRs and muscarinic receptors inhibits a voltage-independent K⁺ current contributing to the resting input conductance (such a voltage-independent current sensitive to 2 mM Mg²⁺-free solutions were used to maintain an amplitude of the inward current comparable with that obtained with 2 mM Mg²⁺).

Involvement of second-messenger pathways

We have previously shown that the reduction in membrane K⁺ conductance in response to the stimulation of mGluRs is prevented when G protein function is disrupted (Gerber and Gähwiler 1994; Guérineau et al. 1994). To date it has not been possible, however, to identify other diffusible cytosolic second messengers involved in the signal transduction process, raising the possibility that G proteins interact directly with K⁺ channels in hippocampal pyramidal cells. Evidence for direct gating of K⁺ channels by G protein components has been found in the hippocampus (VanDongen et al. 1988). Although the present study does not permit conclusions to be drawn concerning the molecular mechanisms by which K⁺ channels are modulated, our findings suggest that an analogous process involving a voltage-dependent interaction between G proteins and K⁺ channels may underlie the effect of 1S,3R-ACPD on a tonic K⁺ current.
Physiological significance

A region of negative slope conductance in the I-V relationship of voltage-gated ionic channels is typical for regenerative phenomena such as the generation of action potentials by the tetrodotoxin-sensitive Na⁺ channels (Hodgkin and Huxley 1952) and the self-amplifying depolarization triggered by Mg²⁺-gated NMDA receptors (Mayer et al. 1984; Nowak et al. 1984). The depolarization-induced 13,3R-ACPD-dependent inward current may be of comparable functional importance. In contrast to NMDA receptors, this mGlur-mediated effect is slower by orders of magnitude, reaching its peak within the time scale of seconds rather than milliseconds. The involvement of mGlur-induced responses in fast synaptic transmission is thus unlikely (Nakanishi 1994). Once activation of mGlus has occurred, however, a persistent instability of membrane potential for prolonged periods of time may accrue, leading to enhancement of small voltage fluctuations around resting potential. Such a mechanism could be important in physiological processes such as long-lasting changes in cellular excitability and persistent modifications of synaptic efficacy as well as in pathological situations such as epileptiform bursting (McDonald et al. 1993; Merlin et al. 1995; Schoepf and Conn 1993).

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REFERENCES


