Group I mGluR Activation Turns on a Voltage-Gated Inward Current in Hippocampal Pyramidal Cells

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INTRODUCTION

Metabotropic glutamate receptors (mGluRs) have been classified into three groups based on structural homology and their link to intracellular second-messenger systems (Nakanishi 1994). Physiological studies suggest that the different subgroups of mGluRs have distinct roles in neuronal signal processing. Activation of group I and III mGluRs causes presynaptic inhibition (Baskys and Malenka 1991; Desai et al. 1994; Vignes et al. 1995). In contrast, activation of group I mGluRs affects postsynaptic function, causing neuronal depolarization and excitation (Bianchi and Wong 1995; Miles and Poncer 1993; Pozzo Miller et al. 1995; Whittington et al. 1995).

In the hippocampus, stimulation of group I mGluRs elicits neuronal population oscillations at the \( \gamma \) (30–50 Hz) and \( \beta \) (12–30 Hz) frequencies. \( \gamma \) oscillations are elicited primarily by an mGluR-induced excitation of inhibitory interneurons (Whittington et al. 1995), whereas \( \beta \) activities and the associated epileptiform afterdischarges are mediated by an excitation of pyramidal cells (Merlin and Wong 1997; Taylor et al. 1995).

METHODS

Slice preparation

Transverse hippocampal slices 400-µm thick were prepared from adult guinea pigs as described previously (Bianchi and Wong 1995) and placed on nylon mesh in an interface recording chamber (Fine Science Tools, BC, Canada). The control solution consisted of (in mM) 157 Na\(^+\), 136 Cl\(^-\), 5 K\(^+\), 1.6 Mg\(^2+\), 2 Ca\(^2+\), 26 HCO\(_3\)\(^-\), and 11 d-glucose. In some experiments, Mn\(^2+\)-containing solutions were used. These solutions had added Mn\(^2+\) (1.5 mM) and reduced Ca\(^2+\) (0.5 mM). Perfusion media were bubbled with 95% O\(_2\)-5% CO\(_2\) to maintain the pH near 7.4, and the temperature was at 34–36°C.

Electrophysiological recordings

Intracellular recordings of CA3 neurons were carried out using an Axoclamp 2A amplifier (Axon Instruments; Foster City, CA). Electrodes were pulled with thin-wall glass tubing and had resistance of 30–40 MΩ when filled with K-acetate (2 M) solution. Voltage and current signals were digitized and stored in an Intel 386-based computer using a 12-bit A/D converter controlled by pClamp software (Axon Instruments). Voltage-clamp experiments were performed us-

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ing the single-electrode discontinuous clamp mode. The headstage output was monitored continuously on an oscilloscope, and the switching frequency (4–6 kHz) and gain (0.5–1.0 nA/mV) were adjusted so that the decay of voltage transients was complete between switch cycles. Synaptic stimulation was applied using tungsten bipolar electrodes (100 μs; 0.1–1 mA).

**Pharmacological agents**

To optimize the conditions for studying the mGluR-mediated inward current, 6-cyano-7-nitroquinaxaline-2,3-dione (CNQX, 20 μM), 3-((R,S)-2-carboxyhexapropyl-4-yl)-propyl-1-phosphonic acid (CPP, 20 μM), and tetrodotoxin (TTX, 0.6 μM) were added to the perfusing solution. The mGluR agonists and antagonists (S)-3-hydroxyphenylglycine (3HPG), (S)-3,5-dihydroxyphenylglycine (DHPG), (+)-α-methyl-1-carboxyhexanoylglycine (MCPG), and (S)-4-carboxyhexanoylglycine (4CPG) also were added to the perfusate. About 15–20 min was required for added drugs to equilibrate in the recording chamber. The peak inward current amplitude induced by Group I mGluR agonists was usually reached 45–60 min after the addition of the agonist. This delay is probably caused by the slow development of the inward current after mGluR stimulation. Glutamate receptor ligands were purchased from Tocris Cookson (Ballwin, MO); all the other chemicals were from Sigma (St. Louis, MO).

**Estimation of the reversal potential of \( I_{mGluR(V)} \)**

Maximum outward current (\( I_1 \), indicated in Fig. 2C) activated during a hyperpolarizing pulse (\( V_2 \), indicated in Fig. 2A) is given by

\[
I_1 = (g_1 - g_2)(V_2 - E_{mGluR(V)})
\]

where \( E_{mGluR(V)} \) is the reversal potential for \( I_{mGluR(V)} \) and \( g_1 \) and \( g_2 \) are the membrane conductances at the holding potential (steady-state value) and at the end of the hyperpolarizing pulse, respectively. \( g = g_m + g_{mGluR(V)} \) where \( g_m \) is the voltage–independent input conductance of the cell and \( g_{mGluR(V)} \) is the membrane conductance contributed by the mGluR agonist.

Peak inward \( I_{mGluR(V)} \) (\( I_2 \)) observed after the return of the membrane potential back to the holding level (\( V_1 \)) after the hyperpolarization is given by

\[
I_2 = (g_1 - g_2)(V_1 - E_{mGluR(V)})
\]

Dividing Eq. 1 by Eq. 2 yields

\[
I_1/I_2 = -(V_1 - E_{mGluR(V)})/(V_2 - E_{mGluR(V)})
\]

By rearranging the equation, the reversal potential can be calculated

\[
E_{mGluR(V)} = (I_1/V_1 + I_2/V_2)/(I_1 + I_2)
\]

For the preceding equations to be true, the assumption is that when the membrane potential jumps from rest to a hyperpolarized level or vice versa, the membrane conductance remains unchanged for an instant. This assumption is probably justified because the changes in voltage-dependent conductance occurred slowly compared with the instantaneous current response. For the example illustrated in Fig. 2, the time constant of development of the outward current (\( I_1 \)) at \(-80 \) mV was 543.5 ms and that of the inward current (\( I_2 \)) at \(-45 \) mV was 894.3 ms. \( I_1, I_2, V_1, \) and \( V_2 \) were measured from the data to estimate \( E_{mGluR(V)} \).

Student’s \( t \)-test was applied and differences between data sets were considered to be significant when \( P < 0.05 \).

**RESULTS**

**Effects of group I mGluR agonists on the subthreshold properties of CA3 pyramidal cells**

In CA3 pyramidal cells with resting potentials of \(-60 \) to \(-65 \) mV, bath perfusion of the selective group I mGluR agonists 3HPG (50 μM, \( n = 6 \)) or DHPG (50 μM, \( n = 32 \)) produced depolarization of \( 7–18 \) mV and altered neuronal responses to hyperpolarizing current injection. Before agonist application, current-induced hyperpolarizations developed with an exponential time course (Fig. 1A, Control). In the presence of an mGluR agonist, responses to hyperpolarizing current developed in two steps—an initial fast hyperpolarization followed by a slower second phase (Fig. 1, DHPG). Recovery from hyperpolarization at the end of the current pulse mirrored that recorded at the onset of the hyperpolarization, consisting of an initial fast depolarization followed by a slow phase (Fig. 1, DHPG). The amplitude and duration of the slow phase during repolarization increased in proportion to the amplitude of the hyperpolarization.

**Group I mGluR agonist-induced responses examined under voltage clamp**

Because the subthreshold responses elicited by group I mGluR agonists exhibit voltage- and time-dependent changes, we examined the agonist effects under voltage clamp. All voltage-clamp experiments were carried out in the presence of TTX (0.6 μM) to suppress Na\(^+\) current.

Under the control condition, a hyperpolarizing command pulse from \(-45 \) to \(-80 \) mV elicited a brief surge of inwardly directed capacitive current followed by a sustained inward ionic current (Fig. 2A). At the end of the hyperpolarizing pulse, an outwardly directed surge of capacitive current was observed before the membrane current returned to the holding level (Fig. 2A). The time courses of the capacitive current at the onset and release of the hyperpolarizing pulse were fitted with single exponential functions with time constants of 24.7 and 29.5 ms, respectively.

Addition of the group I mGluR agonist DHPG produced an inward shift of the holding current (Fig. 2B). A hyperpolarizing command pulse elicited the capacitive current and an inward ionic current as in the control condition. However, the inward current was not maintained for the duration of the hyperpolarizing pulse. A slow outward current appeared that was not observed in the control condition (Fig. 2B). At the end of the hyperpolarizing pulse, an initial outward surge of capacitive current was followed by a slow inward ionic current \( I_2 \), returning the current to the holding level. The current responses activated during and after the hyperpolarization could be fitted with biexponential functions. The initial shorter time constants, largely reflecting the capacitive currents, were 24.5 and 32.6 ms for the responses at the onset and release of the hyperpolarization, respectively. These values for the capacitive current are similar to those of the control response. The longer time constants, reflecting the slow ionic current elicited in the presence of the agonist, had values of 543.5 and 894.0 ms for the current responses at the onset and release of the hyperpolarization, respectively.

In the presence of the mGluR antagonists MCPG (0.5–1 mM; \( n = 3 \)) and 4CPG (0.5 mM; \( n = 2 \)) in the bath, the effects of DHPG were prevented.

**Conductance mechanism underlying the hyperpolarization-activated outward current induced by group I mGluR agonist**

The outward ionic current \( I_1 \) observed during the hyperpolarizing pulse may represent the turn-off of an inward current...
FIG. 1. Effects of group I metabotropic glutamate receptor (mGluR) agonist on the intracellular response of a CA3 pyramidal cell to hyperpolarizing current pulses. A, left: control voltage responses (top) to injected current pulses. Right: responses of the same cell to the same current injections as in control after the addition of (S)-3,5-dihydroxyphenylglycine (DHPG; 50 μM) to the perfusing solution. ←, point where the fast responses were followed by the slow responses. B, left: control responses of the same cell displayed on a slower time base. Right: responses of the cell after addition of DHPG. Note the prolonged duration of the slow responses elicited after the hyperpolarization.

FIG. 2. Effects of group I mGluR agonist on a CA3 hippocampal pyramidal cell examined under voltage clamp. A, top: membrane current elicited by a hyperpolarizing command pulse. Data points are presented in dots. Solid traces through the dots represent exponential fits to the data points. Bottom: time course of the command pulse. B, top: current responses in the same cell to the same command pulse in the presence of 50 μM DHPG. Data points are fitted by a biexponential function. C: same record as in B now fitted with single exponentials (solid traces) for the ionic currents developed during and after the hyperpolarizing pulse. In A and B, the fitting lines closely follow the data points and cannot be discerned easily.
of the hyperpolarizing pulse is larger than that at the termination of the pulse.

We measured $I_1$ and $I_2$ of cells in response to a series of hyperpolarizations (Fig. 3). The values of the currents were measured from traces obtained after subtraction of the responses recorded in the presence of the agonist from those recorded in the control condition (Fig. 3, C and D). For any given hyperpolarization, $I_1$ was always larger than $I_2$ (Fig. 3, E and F), indicating that the cell conductance was always larger at the beginning of the hyperpolarizing pulse than at the end. The data indicate that the outward current activated during the hyperpolarization was associated with a conductance decrease, suggesting that group I mGluR stimulation activates an inward current ($I_{mGluR(V)}$) that is turned off by hyperpolarization and turned on by depolarization.

The values of $I_1$ and $I_2$ also allowed an estimation of the reversal potential for $I_{mGluR(V)}$ (see METHODS). For the cell shown in Fig. 3, corresponding $I_1$ and $I_2$ measurements were obtained for seven levels of hyperpolarizations (from $-80$ to $-110$ mV in 5-mV steps) yielding seven values for $E_{mGluR(V)}$. The mean $I_{mGluR(V)}$ reversal potential for this cell was $-12.3 \pm 2847NOVEL GROUP I mGluR-INDUCED INWARD CURRENT

![Fig. 3](http://jn.physiology.org/)

**Fig. 3.** Current-voltage relationship of $I_{mGluR(V)}$: A: current responses of a CA3 pyramidal cell to a series of voltage-clamp hyperpolarizing pulses from $-40$ to $-110$ mV at 5-mV intervals. Holding voltage: $-45$ mV. For display clarity, only responses to $-40$, $-60$, $-80$, and $-100$-mV command pulses are shown. *, 0 holding current. B: current responses of the same cell after the addition of DHPG (50 μM). C: $I_{mGluR(V)}$ time course obtained by subtracting current traces in A from those in B. Single-exponential fits to the curves are shown as solid lines. Time constants of development of the inward current at $-45$ mV after hyperpolarizing pulses to $-60$, $-80$, and $-100$ mV were 852.5, 894.2, and 818.8 ms, respectively. D: illustration of the measurement of $I_1$ and $I_2$ using as an example the exponential fit to the subtracted record of the responses to $-100$ mV (the trace with the largest outward current shown in C). $I_1$ is the maximum outward current activated by the hyperpolarization and is measured as the coefficient of the exponential fit of the data extrapolated to the time of onset of the hyperpolarization. Note that the outward current ($I_1$) represents a turn-off of an inward current (see RESULTS). $I_2$ is the maximum inward current, obtained in a similar manner from the subtracted responses after the hyperpolarizing pulse. $\Delta I_{on}$ and $\Delta I_{off}$ are the differences between the control and DHPG-modified instantaneous currents at the onset and offset of the hyperpolarization, respectively. E: plot of the peak outward $I_{mGluR(V)}$ ($I_2$; ○) elicited by different levels of hyperpolarizations and of the peak inward $I_{mGluR(V)}$ ($I_1$; ●) activated by the turning off of the hyperpolarization for the cell shown in A–D. F: mean current-voltage relationship ($n = 5$ cells) for the peak outward $I_{mGluR(V)}$ ($I_2$; ○) recorded during the hyperpolarizing pulse and the peak inward $I_{mGluR(V)}$ ($I_1$; ●) recorded after the hyperpolarizing prepulse. Error bars = SE. In E and F, the y axis displays the values of $I_1$ and $I_2$ at various voltage levels normalized to the maximum $I_1$ and $I_2$, respectively.
3.0 mV. The mean reversal potential value obtained in this way for a total of five cells was $-9.8 \pm 1.1 \text{ mV}$.

In addition to the activation of $I_{\text{mGluR(V)}}$, stimulation of group I mGluRs also produced a voltage-independent decrease in the baseline conductance of the cell. The group I mGluR agonist reduced the instantaneous currents elicited both at the onset ($\Delta I_{\text{on}}$) and release ($\Delta I_{\text{off}}$) of the hyperpolarizing pulses compared with the control response. This was reflected in subtracted traces where the instantaneous currents ($\Delta I_{\text{on}}$ and $\Delta I_{\text{off}}$) were always outward (e.g., Fig. 3D). Figure 3D also shows that $\Delta I_{\text{off}}$ is larger than $\Delta I_{\text{on}}$. This observation is consistent with the hypothesis that an outward current is turned off by the hyperpolarizing pulse.

**Relationship between $I_{\text{mGluR(V)}}$ and $I_{\text{Ca}}$**

CA3 pyramidal cells have robust voltage-gated Na$^+$ and Ca$^{2+}$ conductances. Because the preceding experiments were carried out in TTX, the contribution of intrinsic Na$^+$ conductance to $I_{\text{mGluR(V)}}$ appears unlikely. We explored a possible relationship between $I_{\text{mGluR(V)}}$ and intrinsic voltage-gated Ca$^{2+}$ currents.

Nimodipine (10 μM), a blocker of L-type calcium channels, was applied to the perfusing solution after group I mGluR stimulation ($n=4$; Fig. 4). The agent blocked the generation of Ca$^{2+}$ spikes (Fig. 4C). The amplitude of $I_{\text{mGluR}}$ suppressed by hyperpolarization was not affected by nimodipine (Fig. 4, A and B). In addition, current-clamp experiments carried out in the same cell showed that the slow hyperpolarization responses induced by group I mGluR agonists persisted in the presence of nimodipine (Fig. 4C, right).

In other experiments, Mn$^{2+}$ (1.5 mM), a nonspecific Ca$^{2+}$ conductance blocker, was added to a solution with reduced Ca$^{2+}$ (0.5 mM) after $I_{\text{mGluR(V)}}$ was elicited. Mn$^{2+}$ blocked Ca$^{2+}$ spikes in the recorded cell without affecting the amplitude of $I_{\text{mGluR(V)}}$. The hyperpolarization-activated outward current induced by DHPG persisted under Mn$^{2+}$ treatment ($n=3$; data not shown).

**Effects of group I mGluR agonists on the baseline conductance of CA3 pyramidal cells**

On occasion, the two effects of DHPG, namely the reduction in cell conductance and the activation of $I_{\text{mGluR(V)}}$, appeared separately during the wash in of the agonist. DHPG first produced a decrease in instantaneous current responses to hyperpolarizing steps (early response; Fig. 5, A(ii) and B). Over time and with continued agonist perfusion, the slowly developing hyperpolarization-activated outward current appeared (delayed response; Fig. 5A(iii)).

**FIG. 4.** Suppression of Ca$^{2+}$ current did not affect the generation of $I_{\text{mGluR(V)}}$. A: current responses to a series of hyperpolarizations. (i): cell was held at $-40 \text{ mV}$ and steps of hyperpolarization at 5-mV increments were applied to the cell. (ii) and (iii): responses of the same cell to the hyperpolarization after indicated drug treatments. B: net $I_{\text{mGluR(V)}}$ generated after DHPG (●) and after DHPG and nimodipine (●). C: current-clamp records of responses to current injection; same cell as shown in A and B. (i): suprathreshold depolarization elicited Ca$^{2+}$ spikes after DHPG treatment (TTX was present in the solution throughout the experiment). (ii): addition of nimodipine (10 μM) blocked Ca$^{2+}$ spike generation (left), but the mGluR-activated slow hyperpolarizing responses elicited by current injection persisted (right).
Figure 5 shows a plot of the current-voltage (I-V) relationship of the cell in response to hyperpolarizing pulses. The I-V relationship for both the control (●) and early (▲) test responses were linear. The two lines cross at about −90 mV, close to the $K^+$ equilibrium potential of the cell. The separation of early and delayed responses was observed in two other cells. Extrapolated reversal potentials obtained from these two cells were −123 and −127 mV.

Activation properties of the group I mGluR-induced current

The data suggest that $I_{mGluR(V)}$ is an inward current activated by depolarization. We examined the activation threshold of the current (Fig. 6). In the presence of DHPG, cells were held at −45 mV. A conditioning pulse of −90 mV was applied for 750 ms to deactivate $I_{mGluR(V)}$ (Fig. 6B). This was followed by a series of 2-s test pulses from −90 to −20 mV in 5-mV increments. Inward current ($I_{mGluR(V)}$) first appeared when the test pulses were at about −75 mV (Fig. 6, D and E). The amplitude of $I_{mGluR(V)}$ progressively increased with increasing levels of test depolarization and peaked between −30 to −20 mV. Once activated, $I_{mGluR(V)}$ persisted for the duration of the depolarization without showing inactivation.

Effects of group I mGluR stimulation on the resting potential and firing pattern of CA3 pyramidal cells

Because $I_{mGluR(V)}$ is a persistent current and it had a threshold close to the resting potential, we observed interesting modulations of the resting potential by group I mGluR agonists. In current-clamp recordings, cells exhibiting stable depolarized resting potentials in the presence of DHPG occasionally shifted their resting potential to a more hyperpolarized level. The hyperpolarized resting potential was brought about by an incomplete recovery of the cell response from a hyperpolarizing pulse (Fig. 7A). Figure 7B shows that an inhibitory postsynaptic potential (IPSP) could reset the resting potential of the cell to a more hyperpolarized level in a manner similar to that elicited by intracellular current injection.

For cells resting at the more hyperpolarized state in the presence of the group I mGluR agonist, depolarizing currents could reset the resting potential to a more depolarized level (Fig. 7C). In such instances, the fast phase depolarization elicited by the applied current was succeeded by a slow phase depolarization usually causing phasic cell firing. After the phasic firing, the membrane potential often stayed at the depolarized level. In six cells examined, bilevel swings of the resting potential ranged from 8 to 19 mV. This phenomenon of bilevel resting potential was not observed in cells under the control condition.

DISCUSSION

The data indicate that stimulation of group I mGluRs activates a novel inward current ($I_{mGluR(V)}$) with a reversal potential at about −10 mV. A distinct property of $I_{mGluR(V)}$ is its voltage dependency: the amplitude of $I_{mGluR(V)}$ increases...
monotonically when the cell is depolarized (Fig. 6). The threshold of activation of \( I_{\text{mGluR(V)}} \) is between 275 and 270 mV and maximum activation of \( I_{\text{mGluR(V)}} \) occurs at about 230 mV. In addition to the activation of \( I_{\text{mGluR(V)}} \), group I mGluR agonists also elicit a voltage-independent reduction in \( K^+ \) conductance.

The low activation threshold of \( I_{\text{mGluR(V)}} \) facilitated our studies of the current. \( I_{\text{mGluR(V)}} \) can be examined using hyperpolarizing pulses from 245 mV. Hyperpolarizing pulses at this range also should activate \( I_q \) (Perkins and Wong 1995), the only other time- and voltage-dependent persistent current operating at this voltage range. Activation of \( I_q \) would produce a slow inward current opposing the development of \( I_{\text{mGluR(V)}} \). Current- (Fig. 1) and voltage-clamp (e.g., Figs. 2 and 3) recordings show that no noticeable time-dependent current developed in response to hyperpolarizing pulses under the control condition. This observation is consistent with previous findings suggesting that \( I_q \) is not prominently expressed in CA3 cells (Bianchi et al. 1999) in contrast to its significant presence in CA1 cells (Perkins and Wong 1995). To further ensure that the properties of \( I_{\text{mGluR(V)}} \) were examined in isolation from other contaminating responses, all the data used for analysis were obtained by subtracting the control responses from those recorded in the presence of an agonist.

**Group I mGluRs activate a voltage-dependent depolarizing current**

There are two major mechanisms by which stimulation of mGluRs has been shown to depolarize hippocampal cells. These are an increase in a nonspecific conductance to cations (Congar et al. 1997; Guérineau et al. 1995) and a decrease in conductance to potassium ions (Charpak et al. 1990; Guérineau et al. 1994; Harata et al. 1996; Lüthi et al. 1997; Stratton et al. 1989). A recent study also shows that group I mGluR stimulation elicited an inward current in basolateral amygdala neurons via an activation of the Na\(^+\)-Ca\(^{2+}\) exchange (Keele et al. 1997). This current differs from \( I_{\text{mGluR(V)}} \) in that it is not

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**FIG. 6.** Activation properties of \( I_{\text{mGluR(V)}} \). A: current responses recorded from a CA3 cell in response to the voltage protocol presented in the inset. From a holding voltage of -45 mV, a conditioning prepulse to -90 mV was delivered and then followed by test pulses to various amplitudes. B: current responses obtained in the presence of DHPG (50 µM). C: time course of \( I_{\text{mGluR(V)}} \) obtained by subtracting current traces in A from those in B. *, beginning and the end of the segment of current responses displayed. The time constant for activation decreased with depolarization. For this cell, \( \tau \) was 1,272.6 ms at -55 mV; 1,023.3 ms at -50 mV; 978.1 ms at -45 mV; 947.3 ms at -40 mV; 952.8 ms at -35 mV; 825.0 ms at -30 mV; 684.1 ms at -25 mV; and 628.2 ms at -20 mV. D: activation curve, consisting of a plot of the maximum amplitude of \( I_{\text{mGluR(V)}} \) (\( I_2 \) in the inset) obtained during the test pulse vs. the different voltage levels of test pulse. Maximum \( I_{\text{mGluR(V)}} \) (\( I_2 \)) for a given test pulse is given by the coefficient of the exponential fit for the current. E: mean activation curve; data were normalized to the respective maximum \( I_2 \) in each cell (n = 5 cells). Error bars = SE. Note that at test pulses below -70 mV \( I_2 \) is in the outward direction (D and E). This reflects the continuing deactivation of \( I_{\text{mGluR(V)}} \), which was activated at the holding potential of -45 mV. The conditioning pulse at -90 mV deactivated the current producing the outward current. The deactivation process was not complete at the end of the conditioning pulse and continued into the test pulses. The presence of this residual deactivation may cause an underestimate of the amplitude of \( I_2 \) at all levels of depolarization.
accompanied by conductance change and is insensitive to voltage (Keele et al. 1997). The mGluR-activated opening of nonspecific cationic channels involves the direct action of G proteins (Pozzo Miller et al. 1995) or intracellular Ca$^{2+}$ increase (Congar et al. 1997) and does not show voltage-gated properties. In contrast, voltage-dependent mGluR-mediated depolarizations have been noted by others (Charpak et al. 1990; Guéritino et al. 1994; Lüthi et al. 1997) and have been attributed to an mGluR-mediated suppression of potassium conductances. The voltage dependency of the response was explained by assuming that the agonist-mediated conductance block itself is voltage dependent and is markedly increased at depolarized potentials (Lüthi et al. 1997). However, the voltage-dependent depolarization induced by group I mGluR activation in our studies is accompanied by a conductance increase and therefore cannot be attributed to a blockade of potassium channels. Instead, the present data indicate that the depolarization is mediated by a novel, voltage-gated inward current that requires cell depolarization for its activation.

A similar dichotomy exists for the explanation of the muscarinic receptor-mediated depolarization. Although a decrease in potassium conductance had been suggested to be a primary action mediated by muscarinic receptors (Benardo and Prince 1982; McCormick and Prince 1986), more detailed examination revealed that the fundamental mechanism for the muscarinic depolarization of cortical neurons involves the activation of a voltage-dependent inward current with a reversal potential near $-15 \text{ mV}$ (Haj-Dahmane and Andrade 1996). The authors suggest that the voltage-dependent properties of the current led to the apparent conductance decrease observed under voltage- or current-clamp measurements.

A recent study by Wu and Barish (1999) showed that group I or group II mGluR agonists suppressed $I_D$ (Wu and Barish 1992) by acceleration of its inactivation. The activation threshold of $I_D$ is depolarized to $-45 \text{ mV}$, thus the effects of mGluR agonists on $I_D$ should not affect most of our study demonstrating the deactivation of $I_{\text{mGluR(V)}}$ by hyperpolarizing pulses from a holding potential of $-45 \text{ mV}$ (Figs. 2–5). The action of mGluR agonists on $I_D$ may introduce overestimation in our measurements of $I_{\text{mGluR(V)}}$ at depolarizing pulses above $-45 \text{ mV}$ (Fig. 6). However the peak activation of $I_D$ is depolarized to $+30 \text{ mV}$, whereas $I_{\text{mGluR(V)}}$ is maximally activated at around $-30$ to $-20 \text{ mV}$. This suggests that the inward current caused by an accelerated inactivation of $I_D$ may not be a major factor affecting the measurement of $I_{\text{mGluR(V)}}$. However, the detailed effects of the mGluR agonist modulation of $I_D$ on the kinetic properties of $I_{\text{mGluR(V)}}$ can only be derived from studies using dendrotoxin and 4-aminopyridine.

Because of the complex structure of hippocampal pyramidal cells, the data obtained in this study will be affected by space-clamp errors. However, these errors may not be a major factor in determining the measurements of the time course of $I_{\text{mGluR(V)}}$ because different amplitudes of $I_{\text{mGluR(V)}}$ appearing at the same membrane potential developed with comparable time constants (Fig. 3), whereas systematic variations in the activation time constant were observed when $I_{\text{mGluR(V)}}$ developed at different levels of depolarization (Fig. 6).

Although we emphasize the existence of a voltage-dependent inward current turned on by group I mGluR activation, we also observed the coexistence of a voltage-independent conductance decrease contributing to the production of mGluR-mediated depolarization. The reduction in cell conductance can occur separately over time from the development of $I_{\text{mGluR(V)}}$ during agonist wash in (Fig. 5) and is expected to amplify the $I_{\text{mGluR(V)}}$-mediated effects on the membrane potential and firing pattern of pyramidal cells.

$I_{\text{mGluR(V)}}$: a novel, voltage-dependent inward current activated by group I mGluRs

Five unique features of $I_{\text{mGluR(V)}}$ distinguish this current from other known voltage-dependent inward currents, including Na$^+$ and Ca$^{2+}$ currents, intrinsic to hippocampal neurons: 1) $I_{\text{mGluR(V)}}$ is pharmacologically distinct from Na$^+$ and Ca$^{2+}$ currents because it persists in the presence of TTX and Ca$^{2+}$ channel blockers (Fig. 4). 2) $I_{\text{mGluR(V)}}$ is noninactivating. In voltage-clamp experiments, after a hyperpolarizing test pulse ($-80 \text{ mV}$) and on the return of the membrane potential to the holding level ($-45 \text{ mV}$; e.g., Fig. 2), the current develops slowly to a maximum in $1 \text{ s}$. This maximum current shows no sign of decay and is maintained for $\approx 10 \text{ s}$, the longest interval at which cells were held at the holding potential in our experiments before another test pulse. The extent to which $I_{\text{mGluR(V)}}$ persists greatly exceeds that demonstrated for intrinsic Na$^+$ and Ca$^{2+}$ conductances. 3) The voltage-dependent
activation and deactivation of $I_{mGluR(V)}$ develops with time constants on the order of several hundred milliseconds—significantly longer than those of the Na\(^+\) and Ca\(^{2+}\) currents. 4) The threshold of $I_{mGluR(V)}$ is more hyperpolarized (between $-75$ to $-70$ mV) than that of other known noninactivating or slowly-inactivating voltage-dependent inward currents. The latter include L-, N-, P-, and Q-type Ca\(^{2+}\) currents, all of which require depolarization beyond $-45$ mV for activation (Bean 1989; Hess 1990; Hillman et al. 1991; Zhang et al. 1993). And 5) $I_{mGluR(V)}$ is not detectable under normal conditions and appears only when group I mGluRs are stimulated.

**Bistable resting potentials associated with the generation of $I_{mGluR(V)}$**

The low-threshold and noninactivation properties of $I_{mGluR(V)}$ allowed it to contribute in an unexpected way to the maintenance of the resting membrane potential. The data suggest that persistent activation of $I_{mGluR(V)}$ sustained the pyramidal cells in a depolarized state. As a result, in current-clamp recordings, hyperpolarizing pulses turned off the persistent $I_{mGluR(V)}$ and produced the slow phase hyperpolarization (e.g., Fig. 1, A and B). Conversely, on release of the hyperpolarization, $I_{mGluR(V)}$ was turned on. Activation of $I_{mGluR(V)}$ caused regenerative depolarization of the cell, eliciting the slow phase depolarization (Fig. 1, A and B). A stable depolarized membrane potential then is attained when the inward $I_{mGluR(V)}$ is balanced by countering outward currents. Intrinsic Na\(^+\) and Ca\(^{2+}\) currents also can have a tonic influence on the resting potential within the range where the activation and inactivation curves overlap (e.g., Hughes et al. 1999). The contribution of these intrinsic currents to the group I mGluR-mediated changes in the resting potential is not significant because robust voltage-dependent shifts in membrane potential still are elicited by hyperpolarizing pulses in pyramidal cells exposed to DHPG when Na\(^+\) and Ca\(^{2+}\) channels are blocked (Fig. 4C). In this way, group I mGluR-mediated effects on pyramidal cells differ from those shown in other preparations (dorsal horn cells, Morisset and Nagy 1996; turtle motoneurons, Swirskis and Hounsgaard 1998), where Ca\(^{2+}\) channel antagonists blocked the responses mediated by mGluRs. Swirskis and Hounsgaard (1998) showed that group I mGluR action on the turtle motoneurons consisted solely of a depolarization associated with a reduction in input conductance and that this response was sufficient to promote regenerative events driven by intrinsic Ca\(^{2+}\) currents.

$I_{mGluR(V)}$ also enables the hippocampal cells to rest at two membrane potentials. Sufficient hyperpolarization can totally deactivate $I_{mGluR(V)}$ and reset the membrane potential to a hyperpolarized level. Physiologically, hippocampal cells can be driven to operate at the hyperpolarized state by inhibitory postsynaptic potentials (Fig. 7).

The properties of $I_{mGluR(V)}$ can be compared with those of the inward current activated by hyperpolarization ($I_{q}$) (Perkins and Wong 1995). Previous studies emphasized the importance of $I_{q}$ in regulating the pacing activities of hippocampal neurons (Maccabberi and McBain 1996; Perkins and Wong 1995). The two features of $I_{q}$ that allowed it to contribute in this manner are its activation at the subthreshold range and its noninactivation properties—properties similarly possessed by $I_{mGluR(V)}$. The major difference between these two currents is that $I_{q}$ is activated by hyperpolarization, whereas $I_{mGluR(V)}$ is activated by depolarization. Accordingly, $I_{q}$ may have a more dominant role in shaping the subthreshold properties of the cell, whereas $I_{mGluR(V)}$ may play a bigger role in regulating the pacing and firing activities of the cell. We have shown previously that via intrinsic and synaptic interactions, group I mGluR agonists induce hippocampal neurons into prolonged epileptiform afterdischarges (Taylor et al. 1995). Additional studies showed that these group I mGluR-mediated effects are long-lasting (Merlin and Wong 1997; Merlin et al. 1998) and may constitute an epileptogenic process. By eliciting periodic prolonged depolarizations of hippocampal pyramidal cells, $I_{mGluR(V)}$ may sustain the prolonged epileptiform afterdischarges. We used group I mGluR agonists to induce the slow inward current and the epileptiform activity. At present there is no information on whether synaptically released glutamate can induce these responses. Experiments involving tetanization of glutamatergic pathways will be needed to establish the duration and extent of synaptic stimulation that is required to induce $I_{mGluR(V)}$ and epileptiform afterdischarges.

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