Opioid Inhibition of Hippocampal Interneurons via Modulation of Potassium and Hyperpolarization-Activated Cation ($I_h$) Currents

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The actions of mu- and delta-opioid agonists (DAMGO and DPDPE, respectively) on GABAergic interneurons in stratum oriens of area CA1 of the hippocampus were examined by using whole-cell voltage-clamp recordings in brain slices. Both agonists consistently generated outward currents of similar magnitude (15–20 pA) in the majority of cells. However, under control conditions, current–voltage (I/V) relationships revealed that only a small number of these cells (3 of 77) demonstrated clear increases in membrane conductance, associated with the activation of the potassium current known as $G_{mV}$. These interneurons also exhibited a slowly activating, inwardly rectifying current known as $I_h$ on hyperpolarizing step commands. $I_h$ was blocked by the extracellular application of cesium (3–9 mM) or ZD 7288 (10–100 μM) but was insensitive to barium (1–2 mM). In an effort to determine whether the holding current changes were attributable to the modulation of $G_{mV}$ and/or $I_h$, we used known blockers of these ion channels (barium or cesium and ZD 7288, respectively). Extracellular application of cesium (3–9 mM) or ZD 7288 (25–100 μM) blocked $I_h$ and significantly reduced the opioid-induced outward currents by 58%. Under these conditions the opioid agonists activated a potassium current with characteristics similar to $G_{mV}$. Similarly, during barium (1–2 mM) application the opioid-induced outward currents were reduced by 46%, and a clear reduction in $I_h$ and the whole-cell conductance was revealed. These data suggest that the opioids can modulate both $I_h$ and $G_{mV}$ in the same population of stratum oriens interneurons and that the modulation of these ion channels can contribute to the inhibition of interneuron activity in the hippocampus.

Key words: delta-receptor; electrophysiology; enkephalin; GABA; hippocampus; opioid receptor; mu-receptor; nonselective cation current; oriens/alveus interneurons; queer current

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Opioid receptors are members of a class of $G_i/G_o$-coupled receptors that inhibit both adenyl cyclase and voltage-dependent $\mathrm{Ca}^{2+}$ channels (VDCCs) in central and peripheral neurons (Childers, 1993; Moises et al., 1994). In addition to these actions, opioids activate a G-protein-coupled inwardly rectifying potassium channel (Girk) in the same populations of GABAergic interneurons and that the modulation of these ion channels can contribute to the inhibition of interneuron activity in the hippocampus.

Although it is likely that opioids play an important role in the modulation of processes like learning and memory, epilepsy, and drug reinforcement via actions on interneurons in the hippocampus (Siggins et al., 1986; Stevens et al., 1991; Xie and Lewis, 1991), there have been few direct studies of opioid effects on these cells. Most have relied, instead, on indirect measures such as GABA-mediated IPSPs/IPS Cs from pyramidal cells (Lee et al., 1980; Nicoll et al., 1980; Cohen et al., 1992; Lupica et al., 1992; Lupica, 1995). To date, studies using direct recordings from CA1 interneurons have examined only the effects of opioids on $K^+$ channels and VDCCs (Madison and Nicoll, 1988; Wimpey and Chavkin, 1991; Lambert and Wilson, 1996). These investigators found that $G_{mV}$ was activated by the $\mu$-selective agonist DAMGO in acutely dissociated CA1/subicular interneurons (Wimpey and Chavkin, 1991) or by a nonselective opioid agonist (β-Ala$^2$-Met$^5$-enkephalinamide) in stratum pyramidale interneurons (Madison and Nicoll, 1988). However, another study found that DAMGO did not activate $G_{mV}$ nor did it inhibit VDCCs in stratum radiatum interneurons (Lambert and Wilson, 1996).

Another conductance that is prominent in hippocampal interneurons found in stratum oriens is known as $I_h$ (Maccarrferri and McBain, 1996). This slowly developing inward cation current is activated by hyperpolarization, is carried by Na$^+$ and K$^+$ ions, and does not inactivate, even with prolonged hyperpolarization (Hallwell and Adams, 1982; Mayer and Westbrook, 1983; Maccaferri and McBain, 1996). Because of these characteristics, $I_h$ probably contributes to the resting membrane potential and the...
generation of rhythmic pacemaker-like depolarizations in central neurons and cardiac cells (DiFrancesco, 1981; Bal and McCormick, 1996; Maccabber and McBain, 1996; Gasparini and Di Francesco, 1997) (for review, see Pape, 1996). Therefore, modulation of \( I_h \) by neurotransmitters would be expected to alter the oscillatory activity of individual neurons and, in turn, the network of cells with which they communicate (Freund and Buzsáki, 1996) (for review, see Pape, 1996). In addition, because of its properties, \( I_h \) might interact with other conductances by opposing the membrane hyperpolarization initiated by inhibitory neurotransmitters. Among the several neuromodulators that modulate \( I_h \) are the opioids, which inhibit this current in peripheral neurons by inhibiting adenylyl cyclase (Ingram and Williams, 1994). However, at this time no studies of the opioid modulation of \( I_h \) in central neurons have been described. In the present investigation we demonstrate that \( \mu \) and \( \delta \) opioid receptor agonists inhibit \( I_h \) and activate \( G_{\text{Tk}} \) in the same hippocampal interneurons, and we postulate that these effects contribute to the sustained inhibition of interneuron activity by the opioids.

**MATERIALS AND METHODS**

**Electrophysiology.** Hippocampal slices were prepared and maintained as previously described (Miller et al., 1997). Briefly, 14- to 30-d-old male Sprague Dawley rats (Sasco, Omaha, NE) were killed by rapid decapitation. Their brains were removed and placed in ice-cold oxygenated artificial CSF (aCSF; see below). Brain slices containing the hippocampus were cut transverse to the anterior–posterior axis at 300 \( \mu \)m nominal thickness, using a vibrating tissue slicer (Technical Products International, St. Louis, MO). Then the slices were suspended on netting in a beaker containing aCSF that was aerated continuously with 95% \( O_2 \)/5% \( CO_2 \) at room temperature. Control aCSF consisted of (in \( \mu \)M): 126 NaCl, 3.0 KCl, 1.5 MgCl\(_2\), 2.4 CaCl\(_2\), 1.2 NaH\(_2\)PO\(_4\), 11.0 glucose, and 26 NaHCO\(_3\), saturated with 95% \( O_2 \)/5% \( CO_2 \). Interneurons were visualized in stratum oriens of area CA1, using a fixed stage upright microscope equipped with differential interference contrast optics and infrared illumination, as previously described in detail (Miller et al., 1997).

Whole-cell recordings were obtained at room temperature (20–23°C) with an Axopatch-200A amplifier (Axon Instruments, Foster City, CA) and electrodes pulled from thick-walled borosilicate capillary tubing (inner diameter, 0.75 mm; outer diameter, 1.5 mm; Sutter Instrument, Novato, CA). The electrodes had resistances of 4–7 \( \Omega \) when filled with (in \( \mu \)M): 125.0 K-glucuronate, 10.0 KCl, 10.0 HEPES, 1.0 EGTA, and 0.1 M KOH, and brought to 270–280 mOsm with de-ionized water. The morphology of the stratum oriens cells was diverse, and the somata and dendrites confined to stratum oriens is consistent with previous descriptions (Lacaille et al., 1987; McBain et al., 2000; Zhang and McBain, 1995). Examples of two biocytin-filled interneurons reconstructed with the use of camera lucida are shown in Figure 1.

**Opioids generate outward currents in stratum oriens interneurons**

Bath application of the selective \( \mu \)-opioid receptor agonist DAMGO (1 \( \mu \)M) or the selective \( \delta \)-agonist DPDPE (1 \( \mu \)M) generated reversible outward currents in the majority of stratum oriens interneurons. Of the 66 interneurons in which DPDPE was tested, 40 (60.6%) responded with outward currents, whereas 24 of 55 (43.6%) neurons that were tested were sensitive to DAMGO. The magnitudes of the outward currents were similar with either agonist (Fig. 2A). Only 12 of 44 neurons (27%) that were tested responded to both agonists.

In neurons throughout the CNS, opioid-generated outward currents reverse near the \( K^+ \) equilibrium potential (\( E_K \)) and usually are associated with an increase in membrane conductance because of the activation of a G-protein-coupled \( K^+ \) channel known as \( G_{\text{Tk}} \) (Williams et al., 1982; Madison and Nicoll, 1988; North, 1989; Wimpey and Chavkin, 1991). However, in the present study, when the effects of DAMGO and DPDPE were examined on current–voltage (\( I/V \)) relationships (using 250 msec voltage steps from –660 to –136 mV) (Fig. 2B), we found that there was either very little change in the whole-cell conductance or a small net decrease, despite the clear change in holding current (control = 7.1 ± 0.6 nS, pooled DPDPE and DAMGO = 6.8 ± 0.7 nS; \( n = 17 \); measured at steady state from the linear portion of the \( I/V \) curves). This is illustrated in Figure 2C, in which both DPDPE and DAMGO produced changes in the \( I/V \) curves during the peak outward currents that were inconsistent
Rents associated with the activation of whether these neurons were capable of generating outward currents and characteristics similar to the hyperpolarization-activated cation current, $I_{h}$, is indicative of the hyperpolarization-activated cation current $I_{h}$, which is known as $I_{h}$ (visible in Fig. 2). Of those eight neurons lacking $I_{h}$, three demonstrated clear outward currents on opioid application, and these currents were more prominent at step potentials more negative than approximately $-75$ mV (Maccabber and McBay, 1996; Watts et al., 1996). Further isolation of these effects on $I_{h}$ was achieved by subtracting $I_{ins}$ from $I_{h}$ at each voltage step, in the presence and absence of the modulator, using the following equation:

$$\text{subtracted } I_{h} = [I_{sw} - I_{ins}] - (I_{sw} - I_{ins})$$

where $I_{sw}$ and $I_{ins}$ are the steady-state and instantaneous currents measured in the presence of the modulator, and $I_{sw}$ and $I_{ins}$ are these currents in the control condition. $I_{sw}$ was inhibited to a greater extent than $I_{ins}$ by both cesium and ZD 7288 (shown for ZD 7288 in Fig. 5B), and the cesium- and ZD 7288-sensitive currents were more prominent at step potentials more negative than approximately $-75$ mV (Fig. 5C).

 Voltage steps greater than $-136$ mV caused a rapid degradation of the recordings and, therefore, were not used routinely. Overall, cesium (3–9 mM) decreased the whole-cell conductance with an increase in cell conductance expected to result from the activation of a $K^{+}$ channel like $G_{i,h}$. To evaluate whether these neurons were capable of generating outward currents associated with the activation of $G_{i,h}$, we compared the actions of the GABA$_A$ receptor agonist baclofen, which is known to activate this conductance (Gahwiler and Brown, 1985; Inoue et al., 1985; Newberry and Nicoll, 1985; Christie and North, 1988), with the opioid agonists in the same interneurons. At a holding potential of $-66$ mV, baclofen (60 $\mu$m) generated outward currents ($25.0 \pm 4.7$ pA, $n = 7$) that were associated with an increase in membrane conductance of $1.7 \pm 0.5$ nS (Fig. 3). In addition, the $E_{rec}$ for the baclofen-induced current was $-91.1 \pm 4.1$ mV, which is close to the calculated $E_{K}$ of $-96$ mV. These data indicated that, whereas both the opioid and GABA$_A$ receptor agonists produced outward currents, only baclofen activated an apparent $K^{+}$ channel associated with an increase in membrane conductance and characteristics similar to $G_{i,h}$ (compare Fig. 3B,C). A small number of the stratum oriens interneurons (8 of 94, 8.5%) exhibited current responses to voltage steps hyperpolarized to approximately $-76$ mV that lacked the prominent inward sag that is indicative of the hyperpolarization-activated cation current known as $I_{g}$ (visible in Fig. 2B). Of those eight neurons lacking $I_{g}$, three demonstrated clear outward currents on opioid application. In each of these cells the IV curves revealed that the opioid-sensitive currents reversed near $E_{K}$ and were associated with an increase in membrane conductance, consistent with the activation of $G_{i,h}$ (Fig. 4).

Characteristics of $I_{h}$ in stratum oriens interneurons

The hyperpolarization-activated cation current, $I_{h}$, is known to activate slowly and displays virtually no time-dependent inactivation during a voltage step (Mayer and Westbrook, 1983; Maccaferri and McBay, 1996; Watts et al., 1996). In addition, in most preparations $I_{h}$ is not active at membrane potentials positive to approximately $-55$ to $-75$ mV, and it typically does not saturate at potentials as negative as $-155$ mV (Maccaferri and McBay, 1996; Watts et al., 1996). The slow activation kinetics of $I_{h}$ suggest that this current should be larger at the end of a 2 sec voltage step, at which point $I_{h}$ is fully active (i.e., steady state, $I_{ss}$), compared with the current observed near the beginning of a hyperpolarizing voltage step (i.e., the instantaneous current, $I_{ins}$), where the contribution of $I_{h}$ is smaller. Similarly, the voltage-dependent nature of $I_{h}$ suggests that the effects of modulators of this current should be seen at membrane voltages within its range of activation (i.e., negative to $-55$ mV). This can be seen in Figure 5 in which the effects of extracellularly applied ZD 7288 (25 $\mu$m) and cesium (3 mM), both blockers of $I_{h}$, have been examined on the instantaneous and steady-state currents. Further isolation of these effects on $I_{h}$ was achieved by subtracting $I_{ins}$ from $I_{sw}$ at each voltage step, in the presence and absence of the modulator, using the following equation:

$$\text{subtracted } I_{h} = [I_{sw} - I_{ins}] - (I_{sw} - I_{ins}).$$

Figure 1. Camera lucida reconstructions of two biocytin-filled CA1 stratum oriens interneurons that were included in the data set shown in Figure 2. The large majority of these neurons had dendritic processes confined to stratum oriens and demonstrated axons projecting to all of the major hippocampal strata. The data shown in Figure 2B were obtained from the cell on the left of this figure.
Figure 2. Effects of selective \( \mu \)-opioid (DAMGO, 1 \( \mu M \); \( n = 12 \)) and \( \delta \)-opioid (DPDPE, 1 \( \mu M \); \( n = 12 \)) agonists on interneurons with somata located in stratum oriens of area CA1 in hippocampal slices. 

A. Time course of mean ± SEM opioid-induced outward currents. All neurons were voltage-clamped at −66 mV. The horizontal bar indicates the duration of the opioid agonist bath application.

B. Current responses obtained from a single interneuron before (Control) and during DAMGO application that was used in the construction of the curves shown in C (right panel). Voltage steps (250 msec in duration, 10 mV increments) from −66 to −136 mV were used to produce these responses. Note the prominent voltage- and time-dependent inward sag in the current responses at progressively hyperpolarized voltage steps.

C. Mean ± SEM effect of \( \delta \)- and \( \mu \)-agonists on current–voltage (\( I/V \)) relationships obtained during the peaks of the holding current changes shown in A. All data were normalized to the largest current response that was obtained by using a 250 msec voltage step to −136 mV (\( I/I_{\text{max}} \)). In this and subsequent figures the points labeled Subtracted (▲) indicate the drug-induced change in current obtained by subtracting the control points from those measured during drug application.
Figure 3. Effects of the μ-opioid agonist DAMGO (1 μM) and the GABA<sub>B</sub> agonist baclofen (60 μM) on a stratum oriens interneuron. A, Time course of DAMGO- and baclofen-induced outward currents in a neuron voltage-clamped at −66 mV. B, Individual current responses obtained before and during baclofen application (arrows) at the indicated voltage steps. C, I/V curve (250 msec steps between −66 and −136 mV) constructed before (Control), during, and −7 min after (Wash) DAMGO application. Note the larger effect of DAMGO on currents produced at more hyperpolarized steps and the complete washout of this effect. D, I/V curve constructed before (Control), during, and after (Wash) baclofen application. The reversal potential for this baclofen-induced current (−100 mV) is near $E_K$ (−96 mV) in these cells. All data are derived from the same neuron, and the gaps in the records in A are attributable to the construction of the I/V curves.
by ~60% (control = 7.1 ± 1.1 nS; cesium = 4.3 ± 0.85 nS; n = 6), indicating that the contribution of \( I_h \) to the total whole-cell conductance was substantial. Consistent with previous reports, the block of \( I_h \) by ZD 7288 began after 4–7 min of superfusion, and a maximal blockade was achieved after 20–50 min (Harris and Constanti, 1995; Maccarelli and McBain, 1996; Gasparini and DiFrancesco, 1997). A complete block of \( I_h \) by cesium was achieved only at concentrations $>3$ mM but was seen much more rapidly (3–5 min) than with ZD 7288 (data not shown). In addition to the alteration in whole-cell conductance, cesium also generated outward currents ranging from 3.5 to 19 pA (mean = 7.5 ± 2.0 pA; n = 8), suggesting that \( I_h \) may be activated partially in some interneurons at a clamp potential of $-66$ mV. These effects of extracellular cesium are consistent with previous reports demonstrating the blockade of \( I_h \) in other preparations (Mayer and Westbrook, 1983; McCormick and Pape, 1990; Bal and McCormick, 1996). The reversal potential of \( I_h \) ($E_h$) was measured by using the method described by Mayer and

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**Figure 4.** The δ-agonist DPDPE (1 μM) activates an apparent K⁺ current in a stratum oriens interneuron lacking \( I_h \). A. Time course of DPDPE-induced outward current in a neuron voltage-clamped at $-56$ mV. B1. Voltage and current traces obtained $-3$ min before DPDPE application. B2. Current responses obtained before and during DPDPE (arrow) application at the largest voltage step ($-126$ mV). Note the absence of the inward current sag (compare with Fig. 2) and the larger current response during DPDPE application. The holding current change was subtracted from the trace obtained in DPDPE to demonstrate more clearly the change in conductance. C. Current–voltage relationships determined in the same neuron as described in A and B. The DPDPE-sensitive current reversed near $E_K$ (predicted = $-96$ mV; measured = $-95.5$ mV) and was associated with an increase in whole-cell slope conductance. Only 8 of 94 (8.5%) stratum oriens neurons showed a similar absence of inward sag in the current response, and only 3 of these 8 neurons responded to opioid agonists with a similar apparent increase in K⁺ conductance.
Figure 5. Effects of extracellularly applied cesium (3 mM) and ZD 7288 (25 μM) on current responses in stratum oriens interneurons. A. Current records obtained during 2 sec hyperpolarizing voltage steps from −66 to −136 mV in the absence (Control) or presence of cesium (A1) or ZD 7288 (A2). B. I/V relationship demonstrating the effect of ZD 7288 on the instantaneous (ins; Control Ins, ▲; ZD 7288, △) and steady-state currents (ss; Control SS, ●; ZD 7288, ○). C. Cesium- and ZD 7288-sensitive I_h obtained by subtracting the instantaneous I/V relationship from the steady-state I/V relationship in the absence and presence of the blocking agent. Note that both cesium and ZD 7288 had a larger effect on the steady-state versus the instantaneous currents and that both inhibited a similar current with an activation threshold near −75 mV. The symbols in B correspond to those shown in A2.
Westbrook (1983). Thus, instantaneous I/V curves were generated by six to eight voltage steps (2 sec) from holding potentials of \(-56\), in which \(I_h\) is minimally active, and \(-96\) mV, in which \(I_h\) is activated substantially. Then the intersection of the extrapolated I/V curves was used as the estimate of \(E_h\). The estimate of \(E_h\) obtained by using this method was \(-33.0 \pm 2.0\) mV \((n = 7)\), which is similar to the value previously reported in these interneurons (Maccalferri and McBain, 1996).

**Opioids inhibit \(I_h\) in stratum oriens interneurons**

When the effects of DAMGO (1 \(\mu\)M) and DPDPE (1 \(\mu\)M) were examined on currents evoked by 2 sec hyperpolarizing voltage steps, it was found that in some neurons, similar to the effects of cesium and ZD 7288, \(I_{in}\) was inhibited to a larger extent than \(I_{in}\) at voltage steps between approximately \(-76\) and \(-136\) mV (Fig. 6B,C). Thus, the opioid-sensitive \(I_h\) currents were similar to those that were sensitive to ZD 7288 and cesium (compare Figs. SC, 6D).

This is also evident in Figure 7, in which the current responses that were inhibited by ZD 7288 (25 \(\mu\)M), cesium (3 mm), and DPDPE (1 \(\mu\)M) are shown \((V_{hold} = -66\) mV). Here it can be seen more clearly that the current inhibited by ZD 7288 and cesium is similar in its kinetic activation and voltage dependence to that inhibited by DPDPE. Exponential time constants (see below) fit to these currents at the largest hyperpolarizing voltage steps also indicate that the time course of these inhibited currents was similar (Fig. 7, legend). This suggests that all of these agents inhibited a current possessing the same temporal characteristics as \(I_h\). In contrast, the currents that were modulated by baclofen (60 \(\mu\M) are clearly different from those modulated by the inhibitors of \(I_h\) (Fig. 7D).

Because a previous report has suggested that the inhibition of \(I_h\) by baclofen may be secondary to \(G_{ik}\) activation (Watts et al., 1996) and because it was unclear whether the modulation of \(G_{ik}\) or \(I_h\) was responsible for the opioid-induced outward currents in the present study, barium was used to block the opioid activation of \(G_{ik}\) and to permit an assessment of opioid effects on \(I_h\) in relative isolation. These experiments, and those using cesium (below), were conducted in the presence of the Na\(^+\) channel blocker tetrodotoxin (TTX) to enhance the stability of the recordings and to determine whether the effects of DPDPE and DAMGO were direct. Outward currents were generated reliably by the opioids in the presence of TTX, whether administered with barium or cesium (below). Alone, bath application of barium (1–2 mm) caused a decrease in membrane conductance of 1.8 \pm 0.5 nS \((n = 8)\) (Fig. 8B). The \(E_{rev}\) of the current that was inhibited by barium (measured at steady state, using 2 sec voltage steps) was \(-91\pm3.2\) mV, suggesting that, in agreement with previous reports, barium blocked voltage-dependent K\(^+\) channels (e.g., \(I_{IR}\)) (Hille, 1992), and \(I_h\) was, in large part, unaffected (Hilliwell and Adams, 1982; Mayer and Westbrook, 1983; Maccalferri and McBain, 1996). To evaluate the effects of barium on the opioid response, we established sensitivity to DPDPE and DAMGO; then we washed the opioid from the preparation and applied it again in the presence of barium (Fig. 8A). Wherever possible, the effect of the opioid also was evaluated after barium was washed from the preparation (Fig. 8A1). Barium was found to have two major effects on the opioid response. First, in every cell, barium significantly reduced the amplitude of the outward currents generated by DPDPE and DAMGO (Fig. 9A). However, these currents were reduced only to 45.8 \pm 8.8\% of control \((p < 0.05; \text{paired Student’s} \ t\ \text{test}; n = 5)\) (see Figs. 8A1, 11). Second, when the effects of the opioids were evaluated by the use of I/V curves in the presence of barium, it was found that they caused a decrease in the remaining whole-cell conductance in every cell that was tested, with a mean \pm SEM reduction of 24.4 \pm 5.7\% \((n = 8)\) (Figs. 8D, 9B). The reduction in \(I_h\) caused by the opioids in barium was manifest as a 55.2 \pm 7.2\% decrease at \(-86\) mV and a 29.3 \pm 9.8\% decrease at \(-136\) mV \((n = 8)\). In addition, the shape of the subtracted opioid-sensitive current was similar to that obtained for \(I_h\), as defined in the ZD 7288 and cesium experiments (see Fig. SC).

The effect of the opioid agonists on the time constant of \(I_h\) activation also was determined in a subset of opioid-sensitive interneurons. The activation of \(I_h\) was studied by evoking current responses to voltage steps (2 sec) between \(-86\) and \(-136\) mV from a holding potential of \(-66\) mV. Then the inward sag associated with \(I_h\) activation was fit by using exponential functions of the form:

\[
I_t = I_{ss} + I_{ss}e^{-t/\tau}
\]

where \(I_t\) is the amplitude of the current at time \(t\), \(I_{ss}\) is the steady-state current measured at the end of a 2 sec voltage step, \(I_h\) is \(I_{ss}\), \(t\) is the time constant of activation. The rate of \(I_h\) activation demonstrated strong voltage dependence, with control time constants of 0.551 \pm 0.134 sec at the \(-96\) mV voltage step and 0.196 \pm 0.036 sec at the \(-116\) mV voltage step \((n = 8)\). The opioid agonists significantly \((p < 0.001; \text{paired} \ t\ \text{test})\) slowed the activation of \(I_h\), such that the time constants were 1.36 \pm 0.122 sec at \(-96\) mV and 0.45 \pm 0.059 sec at \(-116\) mV. Collectively, these results suggest that, when \(G_{ik}\) was blocked by the addition of barium, the opioids consistently decreased the whole-cell conductance and slowed the rate of \(I_h\) activation. In addition, the persistence of the opioid effect on \(I_h\) in the presence of TTX suggests that it resulted from the direct stimulation of opioid receptors located on the interneurons.

**Opioids activate K\(^+\) channels in stratum oriens interneurons**

In an effort to determine whether the activation of \(G_{ik}\) by the opioids was obscured by the concomitant inhibition of \(I_h\), we examined the effects of DPDPE and DAMGO on stratum oriens interneurons during the blockade of \(I_h\) by extracellular cesium or ZD 7288. A protocol similar to the one described for the barium experiments was used except that, in each case, the concentration of cesium or ZD 7288 was increased until a complete block of \(I_h\) was achieved (cesium, 3–9 mm; ZD 7288, 25–100 \(\mu\)M). At a voltage-clamp potential of \(-66\) mV, cesium inhibited the opioid-induced outward current in every cell that was examined \((n = 6)\) (Fig. 10A). On average, cesium inhibited this response by 58.1 \pm 10.8\% \((p < 0.01; \text{paired Student’s} \ t\ \text{test})\) (Fig. 11), suggesting that the inhibition of \(I_h\) was partly responsible for the opioid-induced change in holding current. In addition, as reported above, the application of cesium alone generated outward currents that were similar in magnitude to those caused by the opioids during barium application. This observation suggests that the inhibition of inward current associated with \(I_h\) by both cesium and the opioids was partially responsible for the holding current changes. When the interneurons were treated with a combination of barium (2 mm) and cesium (3–9 mm), the opioid-induced outward currents were blocked nearly completely \((14.6 \pm 7.5\% \text{ of control}, n = 6)\) (Fig. 11), and there was no effect of the opioids on the I/V relationship.

As reported above, under control conditions the opioid ago-
Figure 6. The δ-opioid agonist DPDPE inhibits $I_h$ in a stratum oriens interneuron. A, DPDPE-induced (1 μM) outward current in a cell voltage-clamped at −66 mV. Gaps in the data record are attributable to the construction of $I/V$ curves. B, Current responses evoked by selected 2 sec hyperpolarizing voltage steps before and during DPDPE (arrow) application. C, Effect of DPDPE on instantaneous and steady-state $I/V$ relationships. Note that, similar to ZD 7288 and cesium (see Fig. 5), DPDPE caused a greater reduction of the steady-state versus the instantaneous current at voltage steps hyperpolarized to approximately −85 mV. D, Effect of DPDPE on $I_h$ isolated by using the procedure described in Results. Note that DPDPE caused a voltage-dependent reduction in this current and that the opioid-induced inhibition was qualitatively similar to that observed with cesium and ZD 7288 (see Fig. 5). Similar effects also were seen with the μ-opioid agonist DAMGO.
larizing voltage steps to obtained under control conditions. In this experiment 500 msec hyperpo-

A previous study has suggested that extracellular cesium can reduce the inward component of the baclofen-activated K⁺ current observed at step potentials more negative than approximately −110 mV without affecting baclofen-induced outward currents (Sodickson and Bean, 1996). Therefore, in the present study the magnitude of the opioid-induced increase in G_{Vrev} in the presence of cesium probably was underestimated. Moreover, the observation that cesium did not alter the outward currents produced by baclofen implies that the opioid-induced outward currents caused by K⁺ channel activation similarly were unaffected by cesium and that the cesium attenuation of the opioid-induced outward currents likely was attributable to the blockade of I_h. These findings, considered together, suggest that the opioids activate a K⁺ conductance and inhibit I_h in the same stratum oriens interneurons and that both of these conductances contribute to the holding current changes seen at a holding potential of –66 mV.

DISCUSSION

The results of this study demonstrate that opioid agonists selective for either μ- or δ-receptors can generate outward currents in hippocampal stratum oriens interneurons. This would result in membrane hyperpolarization and the inhibition of these cells, which is consistent with the effects of opioids on neuronal populations throughout the CNS (Williams et al., 1982; Madison and Nicoll, 1988; North, 1989; Wimpey and Chavkin, 1991; Johnson and North, 1992). However, our results diverge from these previous studies in that this effect is not associated with an increase in membrane conductance and the apparent activation of K⁺ channels under control conditions. Instead, our results indicate that the outward currents produced by the opioid agonists in the absence of channel blockers are associated with a small decrease in conductance. One explanation for these disparate findings is that, in stratum oriens interneurons, either the opioids modulate a different current than that described in other systems or they simultaneously act at more than one ion channel. The large contribution of I_h to the total membrane conductance (Maccaferrì and McBain, 1996) and the previously described actions of the opioids on G_{Vrev} made these currents prominent candidates for further investigation. The present results suggest that concomitant modulation of these ion channels can explain the ambiguous results of these studies.
Figure 8. Blockade of G_{ik} by barium reveals the opioid inhibition of I_h in stratum oriens interneurons. A. Time course of DAMGO- and DPDPE-induced (both at 1 μM) outward currents and inhibition by barium (2 mM). A1, A single experiment in which DAMGO and DPDPE generated outward currents. Barium reversibly reduced the outward current produced by DPDPE application. A2, Another neuron in which both DAMGO and DPDPE produced outward currents. The effect of DAMGO was nearly eliminated by barium in this cell. B, Effects of barium (arrows) on current responses at selected 2 sec voltage steps. The holding current changes were subtracted to illustrate the effect of barium on the whole-cell conductance. The apparent reduction in the steady-state current produced by barium reversed near E_K. C, Effect of DAMGO on the steady-state I/V relationship in the absence of barium (same cell as A2). D, Effect of DAMGO on the steady-state I/V relationship during the application of 2 mM barium. Note the reduction in the whole-cell conductance caused by DAMGO. Similar results were observed with DPDPE.
The properties of $I_{h}$, as it has been described in the present study, are similar to those identified in this and many different neuronal and cardiac preparations (DiFrancesco, 1981; Halliwell and Adams, 1982; Mayer and Westbrook, 1983; McCormick and Pape, 1990; BoSmith et al., 1993; Ingram and Williams, 1994; Harris and Constanti, 1995; Maccaferri and McBain, 1996; Watts et al., 1996; Gasparini and DiFrancesco, 1997) (for review, see Pape, 1996). Among these characteristics are a reversal potential indicative of a mixed $\text{Na}^{+}/\text{K}^{+}$ current, unique voltage and time dependency, and sensitivity to the blocking agents ZD 7288 and cesium. The evidence in support of the modulation $I_{h}$ by the opioid agonists in the present study is derived from several observations, including (1) the shape of the $I/V$ relationship for the opioid-inhibited currents was similar to that obtained for $I_{h}$,
activation appears to modulate only $G_{irk}$ in these cells. This contrasts with previous reports in which $I_h$ was inhibited by baclofen directly or as the indirect result of $G_{irk}$ activation in dopaminergic neurons (Jiang et al., 1993; Watts et al., 1996). In the present study the opioid inhibition of $I_h$ after the blockade of $G_{irk}$ by barium suggests that this action was direct, and not the consequence of $G_{irk}$ activation. In addition, the observation that both GABA_B and opioid receptors could activate $G_{irk}$, whereas only the opioids inhibited $I_h$, suggests that these effects may occur via coupling to different G-proteins. Alternatively, both GABA_B and opioid receptors may activate K⁺ channels via a shared set of G-proteins, but opioid receptors may use a different pool of G-proteins to modulate $I_h$.

Enkephalins are thought to be the endogenous opioid receptor ligands in the CAI region of the hippocampus, where they are synthesized by some interneurons (Gall et al., 1981). Furthermore, axon terminals containing leu-enkephalin have been found apposed to GABA-positive cell bodies, dendrites, and axon terminals in this brain area (Commons and Milner, 1996). Localization of opioid receptors, using antibodies raised against the cloned $\mu$, $\delta$, and $\kappa$-opioid receptors, has shown that only $\mu$- and $\delta$-receptors are expressed in the CAI region of the hippocampus in appreciable concentrations (Mansour et al., 1995, 1996; Commons and Milner, 1997). Moreover, both of these receptors are distributed widely in this brain area, with the $\delta$-opioid receptor found to be associated with GAD-positive interneurons at particularly high levels in stratum oriens (Commons and Milner, 1997). The high selectivity of DAMGO and DPDPE for these receptor subtypes at the concentrations used in this study is well established (Mosberg et al., 1983; Cotton et al., 1985; Goldstein and Naidu, 1989; Lupica, 1995). This information and the observation that only 27% of the interneurons responding to either DPDPE or DAMGO also responded to the alternate agonist suggest that these peptides were selective for their respective receptor subtypes in the present study. In addition, the finding that outward currents were generated in the majority of interneurons by either DPDPE or DAMGO also responded to the alternate agonist suggest that these peptides were selective for their respective receptor subtypes. Together, these data provide anatomical and pharmacological substrates for the previously described physiological actions of the opioids in the hippocampus, including the activation of $G_{irk}$, the activation of an outward rectifier K⁺ current, and the presynaptic inhibition of GABA release (Madison and Nicoll, 1988; Wimpey and Chavkin, 1991; Cohen et al., 1992; Lupica, 1995). All of these opioid actions ultimately diminish the release of GABA from these interneurons, thereby increasing the excitability of the pyramidal cells on which they impinge. The inhibition of $I_h$ by opioid receptors may provide an additional site to reduce the excitability of the interneurons and alter the activity of the hippocampal network.

**Functional significance of $I_h$ and $G_{irk}$ modulation by opioids**

The most reliable effect of the opioid agonists was to induce outward currents in the stratum oriens interneurons. This response could be produced via the activation of a current with a $E_{rec}$ negative to the holding potential (e.g., $G_{irk}$) or by the inhibition of a current with an $E_{rec}$ positive to the holding potential (e.g., $I_h$). The observations that barium and cesium each reduced the opioid-induced outward currents to a similar degree and that their combined application nearly completely blocked
these responses suggest that both $I_h$ and $G_{I\text{tot}}$ contributed to the holding current changes. Moreover, the observation that cesium alone could generate outward currents similar in magnitude to those caused by the opioids in the presence of barium suggests that $I_h$ is activated partially at the clamp potential of $-66 \text{ mV}$ and that the opioids may generate outward currents via the inhibition of these ion channels. Current-clamp experiments in several laboratories have indicated that the blockade of $I_h$ by ZD 7288 or cesium can result in the hyperpolarization of neurons held near the resting membrane potential (Harris and Constanti, 1995; Maccareferri and McBain, 1996; Gasparini and DiFrancesco, 1977). However, although resting membrane potentials were not recorded routinely in the present study, others have reported these values to range between approximately $-52 \text{ mV}$ and $-66 \text{ mV}$ in stratum oriens interneurons (Lacaille et al., 1987; McBain et al., 1994; Bergles et al., 1996). Furthermore, Maccareferri and McBain (1996) demonstrated in these same interneurons that $I_h$ made a substantial contribution to the membrane conductance when the cells were held at $-60 \text{ mV}$, which is likely close to rest. These data suggest the possibility that the inward current contributed by $I_h$ may help to set the resting membrane potentials of these cells at more depolarized values.

In addition to its possible contribution to the membrane potential, the inhibition of $I_h$ by the opioids also would tend to make these GABAAergic interneurons less responsive to excitatory inputs, particularly at hyperpolarized potentials. Also, the unusual property of the activation of this inward current on hyperpolarized influences of inhibitory (i.e., hyperpolarizing) neuromodulators. Thus, the reduction in neuronal excitability caused by the activation initiated by the opioids and other inhibitory modulators might be reversed substantially by $I_h$. Because of this possibility we hypothesize that the concurrent modulation of $I_h$ and $G_{I\text{tot}}$ in the same cells may help to maintain decreased excitability by preventing $I_h$ from returning the membrane potential closer to action potential threshold. These combined actions would ensure that the inhibition of interneuron activity and GABA release initiated by $K^+$ channel activation would not be diminished by the repolarizing influences of $I_h$.

REFERENCES


