Contribution of the Hyperpolarization-Activated Current (I_h) to Membrane Potential and GABA Release in Hippocampal Interneurons

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Lupica, Carl R., James A. Bell, Alexander F. Hoffman, and Patricia L. Watson. Contribution of the hyperpolarization-activated current (I_h) to membrane potential and GABA release in hippocampal interneurons. *J Neurophysiol* 86: 261–268, 2001. Intrinsic GABAergic interneurons provide inhibitory input to the principal neurons of the hippocampus. The majority of interneurons located in stratum oriens (s.o.) of the CA1 region express the hyperpolarization-activated cation current known as I_h. In an effort to elucidate the role of this current in regulating the baseline excitability of these neurons and its participation in the regulation of the release of GABA onto CA1 pyramidal neurons, we utilized whole cell electrophysiological recordings from both populations of cells. In voltage-clamp experiments, hyperpolarization of the interneuron membrane initiated a large inward current with an estimated activation threshold of 51.6 ± 7.6 mV and a half-maximal voltage of −73.0 ± 7.0 mV. This current was blocked by bath application of the I_h inhibitors ZD 7288 (50 μM) or cesium (2 mM). Current-clamp experiments at the interneuron resting membrane potential (−61.3 ± 1.2 mV) revealed a significant hyperpolarization, a decrease in the rate of spontaneous action potential discharge, an increase in the cellular input resistance, and the elimination of rebound afterdepolarizations during blockade of I_h with ZD 7288 (50 μM). The hyperpolarizing effect of ZD 7288 was also substantially larger in interneurons clamped near −80 mV using current injection through the pipette. In addition to neurons exhibiting I_h, recordings were obtained from a small population of s.o. interneurons that did not exhibit this current. These cells demonstrated resting membrane potentials that were significantly more negative (−73.6 ± 5.5 mV) than those observed in neurons expressing I_h, suggesting that this current contributes to more depolarized membrane potentials in these cells. Recordings from postsynaptic pyramidal neurons demonstrated that blockade of I_h with ZD 7288 caused a substantial reduction (~43%) in the frequency of spontaneous action potential-dependent inhibitory postsynaptic currents (IPSCs), without altering their average amplitude. However, miniature action-potential-independent IPSC frequency, amplitude, and decay kinetics were unaltered by ZD 7288. These data suggest that I_h is active at the resting membrane potential in s.o. interneurons and as a result contributes to the spontaneous activity of these cells and to the tonic inhibition of CA1 pyramidal neurons in the hippocampus.

INTRODUCTION

Voltage-dependent ion channels are ultimately responsible for changing the amount or pattern of neurotransmitter released from neurons. However, the contributions that these ion channels make to the process of neurotransmitter release in most neuronal networks remains incompletely understood. Hyperpolarization-activated cation channels that carry inwardly rectifying currents (termed I_f) were first identified in cardiac sinoatrial cells (Brown and DiFrancesco 1980; DiFrancesco 1981) and were subsequently characterized in many different neuronal populations in the CNS (for review, see Pape 1996). In neurons, this inward cation current (known as I_h) is carried by Na^+ and K^+, activates slowly, does not inactivate during prolonged hyperpolarization, and possesses a reversal potential (−30 to −50 mV) that is positive to the neuronal resting membrane potential (Halliwell and Adams 1982; Maccaferri and McBain 1996; Mayer and Westbrook 1983; Svoboda and Lupica 1998). Because of these biophysical properties, these ion channels are proposed to contribute to the neuronal resting membrane potential (RMP), patterns of rhythmic action potential discharge, and may provide a mechanism whereby strong membrane hyperpolarizations are opposed (Maccaferri and McBain 1996; Solomon and Nerbonne 1993; Svoboda and Lupica 1998). Additionally, this current can presynaptically facilitate neurotransmitter release at the crayfish neuromuscular junction (Beaumont and Zucker 2000) and postsynaptically regulate the shape of synaptic potentials during the process of synaptic integration in the mammalian CNS (Magee 1998, 1999).

Hyperpolarization-activated cation channels are represented by the products of at least four genes [termed hyperpolarization-activated cyclic nucleotide-sensitive cation nonselective, HCN1 to -4 (Biel et al. 1999; Gauss et al. 1998; Ludwig et al. 1998; Santoro et al. 1998)]. Furthermore the homomeric ion channels derived from the expression of these genes possess distinct biophysical properties and are differentially sensitive to modulation by cyclic adenosine-3′-5′-monophosphate (cAMP) and the ion channel blocker cesium (Ludwig et al. 1998, 1999; Santoro et al. 1998, 2000). Messenger RNAs for HCN1, -2, and -4 genes are most prominently represented in the CNS where localization studies have revealed a heterogeneous distribution. In particular, HCN1 and HCN2 are expressed at high levels throughout several cortical areas and in the hippocampal formation (Moosmang et al. 1999; Santoro et al. 2000).

Whereas the function of I_h has been well defined in thalamic relay neurons where it regulates neuronal firing patterns (Bal

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and McCormick 1996; Luthi and McCormick 1998; McCormick and Pape 1990b), its role in controlling neuronal activity throughout most of the brain is not well defined. In the hippocampus, I_h has been identified in both principal output neurons (pyramidal neurons) (Gasparini and DiFrancesco 1997; Santoro et al. 2000) and in local circuit GABAergic interneurons (Maccaferri and McBain 1996; Santoro et al. 2000; Svoboda and Lupica 1998). These GABAergic interneurons play an important role in regulating hippocampal activity and output because although comprising a relatively small fraction of the total hippocampal neuronal population (~10%), they each form multiple synapses on hundreds of pyramidal neurons (Freund and Buzsaki 1996). This divergent synaptic arrangement thereby permits these interneurons to synchronize the activity of a large number of principal output neurons and initiate neuronal network oscillations that appear to be important for information processing (Cobb et al. 1995; Whittington et al. 1995). We have previously demonstrated that 90% of the GABAergic interneurons with somata located in stratum oriens (s.o.) of the CA1 region of the hippocampus exhibited I_h currents, as defined both biophysically and by sensitivity to the I_h blockers cesium and ZD 7288 (Svoboda and Lupica 1998). In this study, we also demonstrated that I_h contributed as much as 60% of the whole cell conductance on hyperpolarization of the interneuron membrane and that inhibition of I_h by μ- and δ-opioid receptors could generate outward currents in neurons voltage clamped near the RMP (Svoboda and Lupica 1998). These data suggested that I_h may be active near the RMP of these neurons and therefore may play a significant role in regulating s.o. interneuron excitability, GABA release onto CA1 pyramidal neurons, and ultimately hippocampal output. However, because in these previous studies interneurons were clamped at potentials that were likely negative to their actual RMPs (Maccaferri and McBain 1996; Svoboda and Lupica 1998), the contribution of I_h to the actual interneuron RMP could not be assessed. Therefore the goals of the present study were to determine whether I_h contributes to the RMPs of these s.o. interneurons and to define the role that this current plays in the regulation of GABA release onto pyramidal neurons in the CA1 region of the hippocampus. A portion of these results has appeared in a preliminary report (Lupica et al. 1999).

METHODS

Brain-slice preparation

Hippocampal slices were prepared and maintained as previously described (Miller et al. 1997; Svoboda and Lupica 1998). Briefly, 14- to 30-day-old male Sprague-Dawley rats (Sasco, Omaha, NE or Charles River Labs, Raleigh, NC) were killed by decapitation. Their brains were removed and placed in ice-cold, oxygenated artificial cerebral spinal fluid (ACSF; see following text). Brain slices containing the hippocampus were cut transverse to the anterior-posterior axis at 300 μm nominal thickness using a vibrating tissue slicer (Technical Products International, St. Louis, MO). The slices were then 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 mM) 126 NaCl, 3.0 KCl, 1.5 $MgCl_2$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 11.0 glucose, and 26 $NaHCO_3$, and saturated with 95% O_2-5% CO_2. After ≥60 min of incubation in the ACSF, a single slice was transferred to a recording chamber (~250 μl vol) that was built into the stage of an upright microscope (Carl Zeiss Instruments, Oberkochen, Germany).

Interneuron recordings

Interneuron somata were visualized in the s.o. of area CA1 using a fixed stage upright microscope equipped with differential interference contrast optics and an infrared light source (DIC-IR) as previously described in detail (Dodt and Zieglgansberger 1990; Miller et al. 1997; Svoboda and Lupica 1998; Svoboda et al. 1999). Whole cell recordings were obtained from interneurons at room temperature (20–23°C) using an Axopatch-200A or Axoclamp-2A amplifier (Axon Instruments, Burlingame, CA) and electrodes pulled from thick-walled borosilicate capillary tubing (0.75 mm ID, 1.5 mm OD, Sutter Instrument, Novato, CA). The electrodes had resistances of 4–7 MΩ, when filled with (in mM) 125.0 K-gluconate, 10.0 KCl, 10.0 HEPES, 1.0 EGTA, 0.1 $CaCl_2$, 2.0 Mg^{2+}-ATP, and 0.2 Na^+-GTP (adjusted to pH 7.2–7.4 with 1 M KOH, 270–280 mOsm). All recordings were corrected for an 11.5-mV liquid junction potential measured according to the method of Neher (1992). Series resistance was generally <15 MΩ and was monitored throughout the experiments by measuring the capacitative currents generated by small (−5 mV, 250 ms) voltage steps (or current steps during current-clamp recordings). Cells were rejected from analysis if the series resistance changed by 10–15%. Voltage-clamp protocols were delivered using a pulse generator (AMPI Master 8, Jerusalem, Israel), and signals were acquired using a National Instruments Lab PC 1200 A/D converter (Austin, TX) and the Strathclyde electrophysiology software package (courtesy of Dr. John Dempster, Strathclyde University, Glasgow, UK, http://innovol.sibs.strath.ac.uk/physpharm).

Pyramidal neuron recordings

Action-potential-dependent spontaneous inhibitory postsynaptic currents (sIPSCs), and action-potential-independent miniature IPSCs (mIPSCs) were recorded in cells voltage clamped at −70 to −90 mV using the Axopatch-200A amplifier and whole cell electrodes filled with the following solution (in mM): 125.0 CsCl, 10.0 HEPES, 1.0 EGTA, 0.1 $CaCl_2$, 2.0 Mg^{2+}-ATP, and 0.2 Na^+-GTP, and the quaternary lidocaine derivative QX-314, 2 (pH 7.2–7.4). QX-314 was added to eliminate Na^+-dependent action potentials and to block I_h channels in the pyramidal neurons from which whole cell recordings were made (Perkins and Wong 1995). sIPSCs and mIPSCs were also pharmacologically isolated from excitatory postsynaptic currents (EPSCs) by addition of the glutamate receptor antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM) and D-(−)-2-amino-5-phosphonopentanoic acid (APV, 40 μM) to the ACSF. sIPSCs and mIPSCs were amplified 5- to 10-fold, filtered at 3–5 kHz, and 3 min epochs of data were acquired (at 4–10 kHz) directly onto the hard drive of a personal computer.

Chemicals

Drugs were obtained from the following sources: tetrodotoxin (TTX) and QX-314, Alomone Laboratories (Jerusalem, Israel); DNQX, APV, and CsCl, Sigma (St. Louis, MO); ZD 7288, Tocris Cookson (Ballwin, MO). All drugs and channel blocking agents were made at 100 times their final concentration in de-ionized water and added to the ACSF bathing the slice (flow rate = 2 ml/min) using calibrated syringe pumps (Razel Scientific Instruments, Stamford, CT).

Analysis

The frequency, amplitudes, and kinetic properties of s- and mIPSCs were analyzed using the Mini Analysis software package (v4.3, Synaptosoft, Leonia, NJ, http://www.synaptosoft.com). In addition, averaged s- and mIPSCs were generated by aligning individual events by rise time, and a peak to decay single exponential fit was applied to

each average using the formula $y = A1 * \exp(-x/\tau) + \text{Baseline}$, where *A1* is the peak amplitude and τ is the time constant for decay.

In some cases, hyperpolarization-activated currents that were sensitive to ZD 7288 were plotted against the step voltage and fitted using the Boltzmann equation

$$I = I_{max}/\{1 + \exp[V_m - V_{1/2}]/s\}$$

where I_{max} is represented by the maximal current amplitude, $V_{1/2}$ is the voltage at which the current is one half of its maximal level, and *s* is the constant slope factor.

Group data are presented as the means ± SE in all cases. Drug-induced changes in cumulative s- and mIPSC amplitude and interevent interval distributions were analyzed for statistical significance using the Kolmogorov-Smirnov (K-S) test (Mini Analysis v4.3), and a conservative critical probability level of $P < 0.01$. All other statistical tests, including *t*-tests and ANOVAs, were performed using a critical probability of $P < 0.05$ (Prism version 3.0, GraphPad Software, San Diego, CA). Post hoc analyses (Newman-Keuls test) were performed only when an ANOVA yielded a significant ($P < 0.05$) main effect.

RESULTS

Properties of I_h in hippocampal stratum oriens interneurons

As described previously, membrane hyperpolarization under voltage-clamp conditions produced a slowly activating inward current in the majority of interneurons possessing somata located in s.o. of the CA1 region of the hippocampus (Maccaferri and McBain 1996; Svoboda and Lupica 1998). In interneurons voltage clamped at −40 mV, the inward current reached a maximum when the membrane was stepped to approximately −90 to −110 mV, and its amplitude did not decay during a 1.5-s voltage step (Fig. 1, *A* and *C*). This inward current was blocked by ZD 7288 (50 μM; Fig. 1), and cesium (2 mM, not shown); both of which have been shown to inhibit I_h in these neurons (Maccaferri and McBain 1996; Svoboda and Lupica 1998), and the cardiac equivalent of this current (I_f) in sino-atrial pacemaker cells (BoSmith et al. 1993). The effect of ZD 7288 was time dependent, reaching a maximum in approximately 5–7 min, and was relatively selective for I_h since the steady-state (ss) current measured near the end of 1.5-s constant voltage step was reduced to a much larger extent than the instantaneous current (ins) measured near the beginning of the voltage step (Fig. 1, *C* and *D*). Because of this relationship, I_h amplitude was measured by subtracting I_{ins} from I_{ss} at each hyperpolarizing voltage step (Fig. 1*D*) as previously described (Svoboda and Lupica 1998).

Contribution of I_h to membrane potential in s.o. interneurons

The hyperpolarization-activated current was not routinely detected at membrane potentials near the voltage-clamp hold-

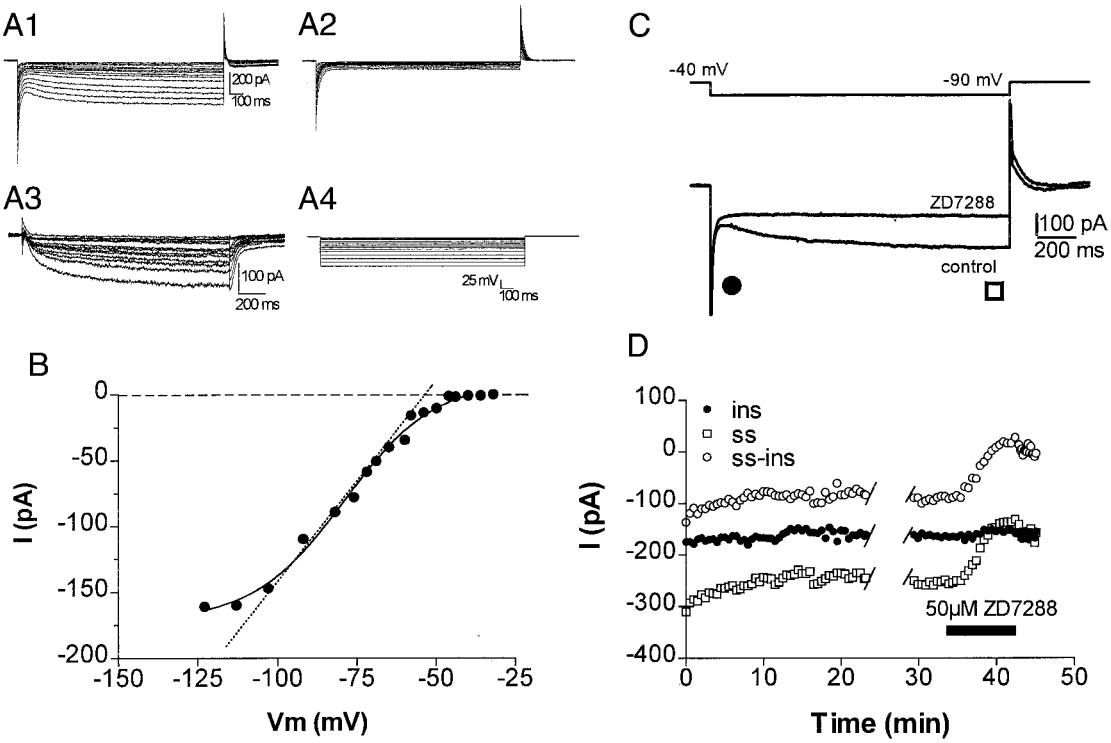

FIG. 1. Properties of I_h in stratum oriens (s.o.) interneurons. *A*: currents recorded during a series of 1.5-s hyperpolarizing voltage steps (*A4*) under control conditions (*A1*) and at the end of a 10-min application of ZD 7288 (50 μM, *A2*). *A3*: the ZD 7288-sensitive currents were obtained by digitally subtracting traces obtained in ZD 7288 (*A2*) from control traces (*A1*). Leak subtraction was not applied to the currents. *B*: plot of the peak ZD 7288-sensitive current vs. the membrane potential (V_m) during hyperpolarizing voltage steps. All points were fit using the Boltzmann equation. The membrane potential at which the ZD 7288-sensitive current was half-maximal ($V_{1/2}$) was −79.1 mV, the maximal current was −176.1 pA, and the slope factor was 14.3 mV. $V_{hold} = -40$ mV. The points between −90 and −70 mV were also fit using linear regression (- - -) to estimate the I_h activation threshold (also see Kilb and Luhmann 2000). *C*: traces demonstrating the effect of ZD 7288 on the instantaneous (●) and steady-state (□) currents during a single voltage step to −90 from −40 mV in another s.o. interneuron. *D*: time course of the effect of ZD 7288 on the responses shown in *C*. The instantaneous and steady-state currents, plotted against time, are shown using the same symbols as in *C*. The I_h current, isolated by subtracting the instantaneous from the steady-state current, is also shown (○). Note that I_h is stable over time and that ZD 7288 selectively reduced the steady state current.

ing potential of −40 mV, suggesting that its activation threshold was at a more negative value (Fig. 1*A*). In an effort to estimate the activation threshold for I_h, the peak amplitude of the ZD 7288-sensitive component of the inward current was plotted against the membrane potential (V_m) at various hyperpolarized voltage steps. The linear portions of these curves (4–5 points between −70 and −90 mV) were then fit using linear regression, and the activation thresholds estimated from the *x* intercept (Kilb and Luhmann 2000). Using this analysis, the activation threshold for I_h was estimated to be −51.6 ± 7.6 mV (n = 5; Fig. 1*B*). Since the mean RMP of another group of s.o. interneurons, under identical recording conditions, was found to be −61.3 ± 1.2 mV (n = 13) and the reversal potential of I_h in these cells is approximately −30 mV (Maccaferri and McBain 1996; Svoboda and Lupica 1998), it is likely that I_h was active at rest and thereby may have exerted a tonic depolarizing influence on these neurons. To test this hypothesis, we recorded from s.o. interneurons at their RMP (i.e., no holding current applied) under current-clamp conditions in the presence of tetrodotoxin (TTX, 500 nM), to block spontaneous action potential discharge, and applied ZD 7288 (50 μM). Application of ZD 7288 for 10 min caused a significant hyperpolarization (to −66.6 ± 1.4 mV, $P < 0.01$, paired *t*-test), greatly increased the cellular input resistance measured at steady state and eliminated the rebound action potential (i.e., the afterdepolarization) (Luthi and McCormick 1998; Pape 1996) commonly observed at the termination of the current step (Fig. 2*A*). To determine whether this hyperpolarization was sufficient to decrease the rate of action potential discharge, we recorded from a separate group of s.o. interneurons in the absence of TTX pretreatment. As reported in previous studies (Maccaferri and McBain 1996; Svoboda and Lupica 1998), the majority (i.e., 10 of 13 in the present study) of s.o. interneurons were spontaneously active. Furthermore, when ZD 7288 (50 μM) was applied, the frequency of action potential discharge was significantly reduced in every spontaneously active cell (n = 10; Fig. 3). These data suggest that I_h contributes to baseline s.o. interneuron excitability.

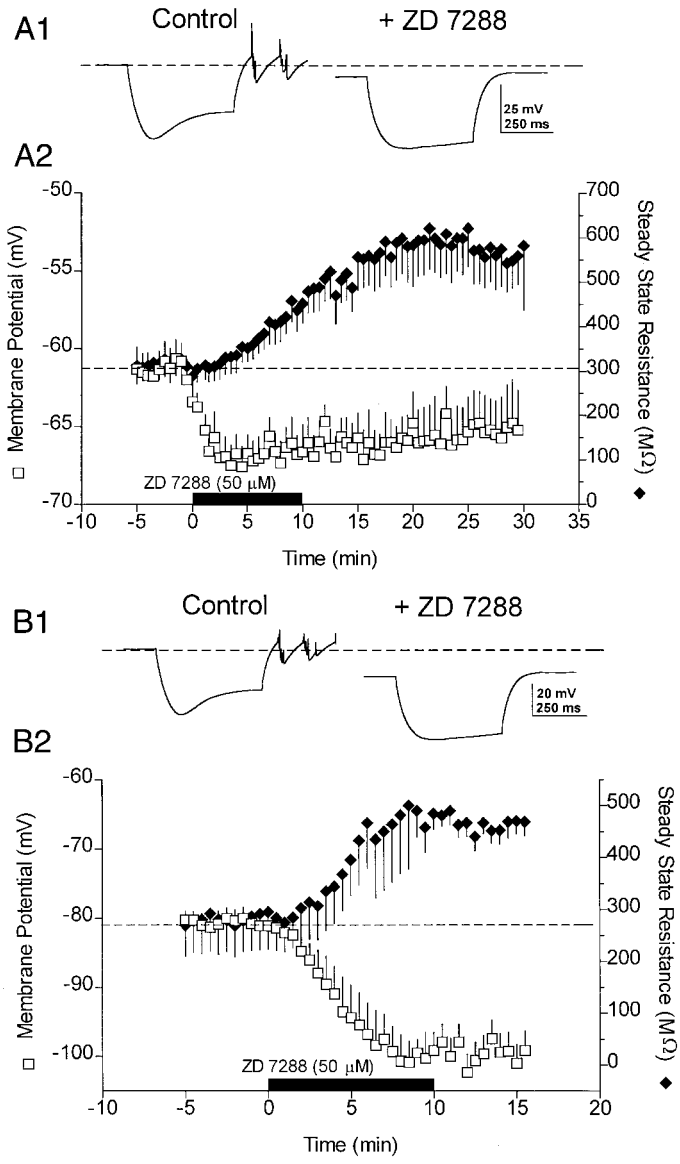

FIG. 2. Effects of ZD 7288 (50 μM) on membrane potential (□) and input resistance (♦) in s.o. interneurons under current-clamp conditions. ZD 7288 caused a significant hyperpolarization, increased the input resistance of interneurons recorded at the resting membrane potential (RMP, *A2*, n = 13), decreased the hyperpolarization-activated voltage sag (*A1*), and eliminated the rebound depolarization observed at the termination of a 0.2-nA hyperpolarizing step (*A1*). The hyperpolarizing effect of ZD 7288 was much larger in neurons that were current clamped near −80 mV (n = 7) than those examined at the RMP (compare *A2* and *B2*, noting the difference in ordinate axis scale). Each point in *A2* and *B2* represents the mean ± SE response for 7–13 interneurons. - - -, the mean RMP and input resistance prior to ZD 7288 application. The shorter duration of the recordings in *B2* was due to an increase in the instability of the recordings following the ZD 7288-induced hyperpolarization, only in the neurons held near −80 mV.

In addition to contributing to the RMP, another of the proposed roles of I_h is to offset strong membrane hyperpolarizations and to return the membrane potential to values closer to action potential threshold (Pape 1996; Svoboda and Lupica 1998). This is because the hyperpolarization-activated inward current is larger at more negative membrane potentials, thereby exerting a stronger depolarizing influence. Because of this property blockade of I_h by ZD 7288 should result in a larger hyperpolarization of the membrane potential in cells that are more strongly inhibited. To test this, a separate group of s.o. interneurons was current clamped to approximately −80 mV, using current injection through the pipette. Then ZD 7288 (50 μM) was applied for 10 min while measuring the membrane potential and cellular input resistance. In these neurons, ZD 7288 caused a much larger hyperpolarization (−99.6 ± 2.9 mV, $P < 0.001$, ANOVA) than that seen in the cells recorded at RMP and again blocked the afterdepolarization normally seen on termination of strong hyperpolarizing current pulses (Fig. 2*B*). Blockade of I_h by ZD 7288 also resulted in a substantial increase in the input resistance that was not significantly different from that measured in the neurons recorded at rest (Fig. 2*B*). These data suggest that I_h is active near the RMP of s.o. interneurons and that it exerts a strong repolarizing influence on hyperpolarized neurons.

The preceding data indicated that tonic activation of I_h contributes to setting the RMP of s.o. interneurons at more depolarized levels. Therefore interneurons lacking I_h should exhibit more hyperpolarized RMPs and should also be insensitive to the effects of ZD 7288. Approximately 10% of all interneurons with somata located in s.o. do not exhibit the membrane sag on hyperpolarization that is associated with I_h (Svoboda and Lupica 1998). We recorded from a sample of

these neurons ($n = 5$) in the present study and found that their RMPs were significantly hyperpolarized (-73.6 ± 5.5 mV) when compared with the majority of s.o. interneurons that exhibited I_h (-61.3 ± 1.2 mV, $n = 13$, $P < 0.01$; Fig. 4). In addition, the interneurons lacking I_h were completely insensitive to ZD 7288 and did not exhibit afterdepolarizations (Fig. 4).

Contribution of I_h to spontaneous GABA release onto CA1 pyramidal neurons

A previous anatomical study in this laboratory demonstrated that a portion of hippocampal interneurons with somata located in s.o. possessed axons that heavily innervated CA1 pyramidal neuron somata (Svoboda et al. 1999). This suggested that this population of cells might provide strong GABAergic inhibition of CA1 pyramidal neurons and thereby contribute to the tonic modulation of their output. In an effort to determine whether I_h contributes to the tonic inhibition of CA1 pyramidal neurons in hippocampal slices, we measured spontaneously occurring IPSCs in these cells under control conditions, and during blockade of I_h by ZD 7288. sIPSCs represent the postsynaptic response to spontaneously released quanta of GABA acting at $GABA_A$ receptors (e.g., see Hoffman and Lupica 2000; Lupica 1995) and are thereby thought to reflect the activity of presynaptic GABAergic interneurons. In the absence of Na^+ channel blockade with TTX (see following text), sIPSCs are composed of both action-potential-dependent and -independent populations. Previous studies have also shown that the frequency of sIPSCs can be modified by neuromodulators that regulate the presynaptic RMP (e.g., see Miller et al. 1997). sIPSCs measured in CA1 pyramidal neurons voltage clamped at -70 to -90 mV using symmetrical concentrations of Cl^-, and in the presence of ionotropic glutamate receptor antagonists (DNQX, 10 μM; APV, 40 μM), demonstrated baseline frequencies of 5.8 ± 0.6 Hz (range = 3.1–13.0 Hz, $n = 11$), and baseline amplitudes of -23.5 ± 1.0 pA (range = -11.9 to -37.4 pA, $n = 11$). These values compare favorably with those reported previously by this and other laboratories in the same preparation (Cohen et al. 1992; Hoffman and Lupica 2000; Lupica 1995; Miller et al. 1997). Addition of ZD 7288 (50 μM) to the superfusion medium resulted in a significant decrease in sIPSC frequency, in every cell tested ($P < 0.001$, K-S test), that became maximal after ~7–10 min. The average frequency of sIPSCs measured 10 min after beginning ZD 7288 superfusion was 3.3 ± 0.2 Hz (range = 1.2–8.4 Hz, $n = 11$), representing a decrease of ~43% (Fig. 5; $P < 0.001$, paired t-test). In contrast to the robust inhibition of sIPSC frequency, blockade of I_h by ZD 7288 did not result in a significant change in mean sIPSC amplitude (21.1 ± 0.7 pA, $P > 0.05$, $n = 11$, paired t-test; Fig. 5, *B* and *C*), despite the observation that significant reductions were observed in 2 of 11 neurons when the cumulative amplitude distributions were analyzed using the K-S test ($P < 0.001$). Together, these data suggest that the blockade of I_h in GABAergic interneurons by ZD 7288 resulted in a decrease in GABA release onto CA1 pyramidal neurons.

mIPSCs recorded during superfusion with TTX (500 nM) were also measured in a different group of CA1 pyramidal neurons to determine whether I_h plays a role in supporting

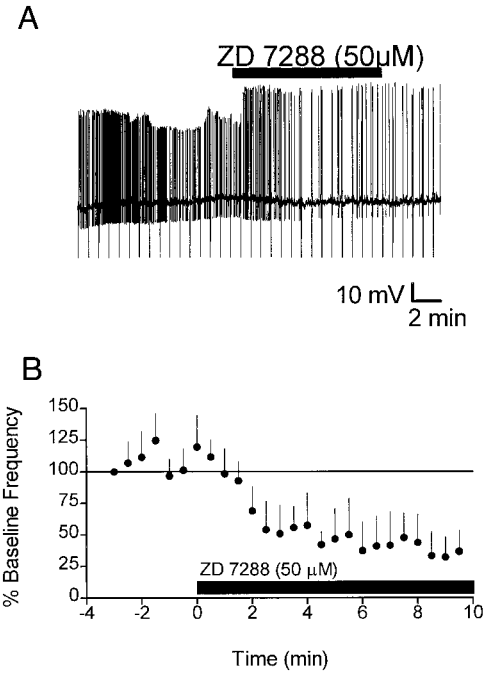

FIG. 3. Effects of ZD 7288 on the frequency of action potentials in s.o. interneurons recorded at the resting membrane potential. *A*: chart recording of the effect of ZD 7288 (50 μM) in a single interneuron during whole cell recording. Note the decrease in the number of spontaneous action potentials (upward pen deflections) during application of ZD 7288. This effect was seen in every spontaneously active neuron ($n = 10$). The downward deflections and the regularly spaced upward deflections represent membrane responses to current injection used to monitor input resistance. The amplitudes of the action potentials are truncated due to the slow frequency response of the chart recorder. *B*: mean ± SE effect of ZD 7288 on relative action potential frequency in a group ($n = 4$) of s.o. interneurons. Data were normalized to the baseline action potential frequency in each cell. Note the pronounced inhibition of spontaneous activity by ZD 7288.

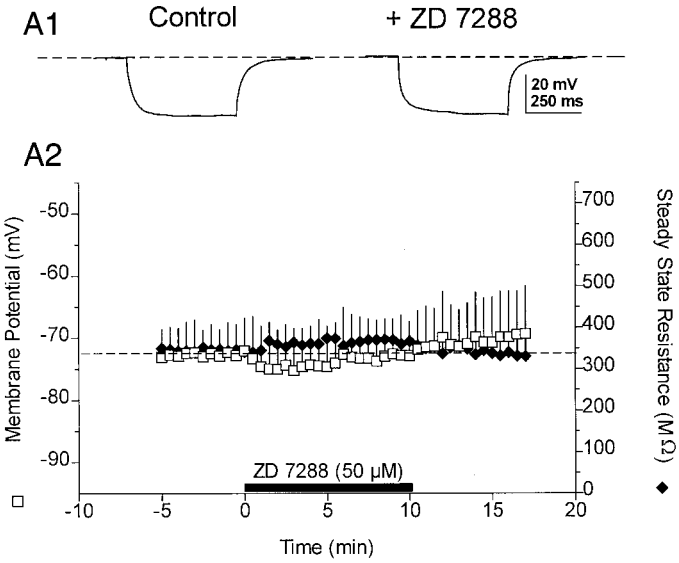

FIG. 4. Stratum oriens interneurons ($n = 5$) lacking I_h exhibit significantly larger RMPs than those possessing this current. *A1*: membrane response to -0.2-nA steps under control conditions and during ZD 7288. Note the lack of the membrane relaxation seen in the majority of these neurons (e.g., compare with Fig. 2*A1*) and the insensitivity to ZD 7288 (i.e., compare Fig. 4 with Fig. 2). *A2*: time course of the effect of ZD 7288 in s.o. interneurons lacking I_h. - - -, the control RMP and input resistance prior to ZD 7288 application. Note that the RMP and the resting input resistance were unaltered by ZD 7288.

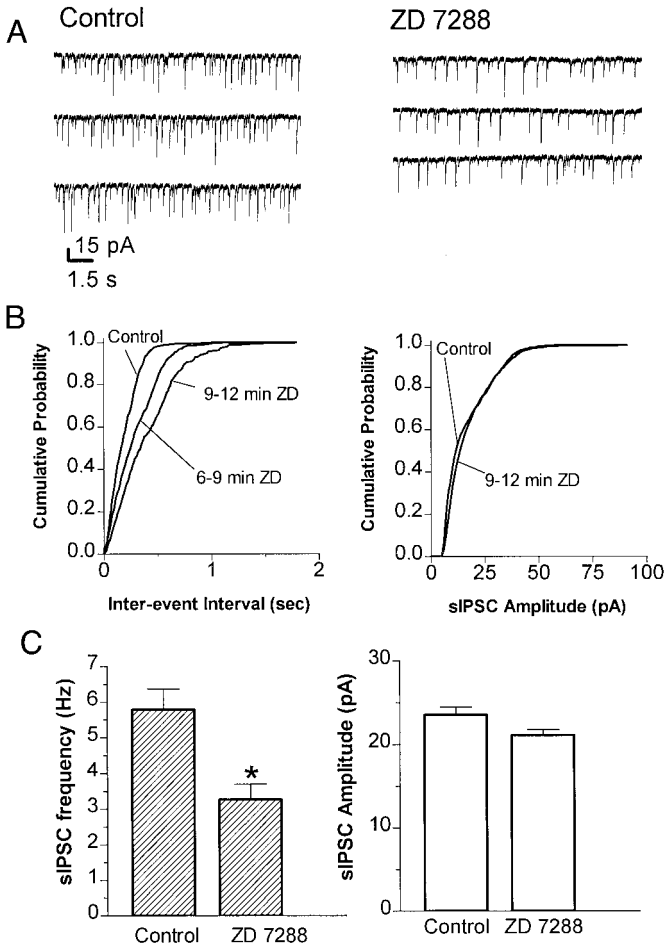

FIG. 5. Blockade of I_h by ZD 7288 (50 μM) decreases spontaneous GABAergic synaptic transmission in CA1 pyramidal neurons. *A*: consecutive traces (15 s each) obtained under control conditions and during the 9th to 12th minute of ZD 7288 application. *B*: cumulative inter-event interval and amplitude histograms illustrating the effect of ZD 7288 on spontaneous GABAergic IPSCs (sIPSCs). Note that there is a time-dependent lengthening of the intervals between sIPSCs during application of ZD 7288 (i.e., a decrease in frequency). Both of these inter-event interval distributions (obtained during the 6th to 9th and the 9th to 12th min of ZD 7288 application) were significantly different from the control distribution ($P < 0.001$, K-S test). Also note the absence of an effect of ZD 7288 on the sIPSC amplitude (*right*). *C*: group data illustrating the effect of I_h blockade on sIPSC frequency and amplitude in CA1 pyramidal neurons. All recordings were performed in the presence of ionotropic glutamate receptor antagonists. *$P < 0.01$, paired *t*-test ($n = 11$).

quantal neurotransmitter release in the mammalian CNS. Recording conditions were similar to those used to measure sIPSCs except that TTX was added to the superfusion medium for ≥15 min prior to application of ZD 7288. Previous studies in this laboratory have demonstrated that this protocol is sufficient to completely eliminate stimulation-induced neurotransmitter release and to completely block action potential generation in CA1 pyramidal neurons (Hoffman and Lupica 2000; Lupica 1995). Consistent with these previous studies, TTX caused a large decrease in the frequency (1.2 ± 0.2 Hz, range = 0.3–1.9 Hz, $n = 15$) and amplitude (17.7 ± 0.5 pA, range = 10.8–25.3 pA, $n = 15$) of mIPSCs (Fig. 6). However, when mIPSCs were measured after 10 min of superfusion with ZD 7288, significant changes in average frequency (1.3 ± 0.2 Hz, range = 0.2–2.2 Hz, $n = 15$, $P > 0.05$) or amplitude (18.8 ± 0.5 pA, range = 14.0–24.4 pA, $n = 15$, $P > 0.05$) were not observed (Fig. 6). Similarly ZD 7288 had no effect on the decay time constants of the mIPSCs (control = 19.4 ± 0.2 ms, ZD 7288 = 19.8 ± 0.1 ms).

DISCUSSION

The RMP of central neurons is established through interactions among a variety of tonically active inward and outward currents. The findings of the present study suggest that the inward current, I_h, plays a significant role in setting the RMP and baseline level of excitability of hippocampal GABAergic interneurons found in the s.o. of area CA1. This conclusion is supported by several pieces of data in the present study. First, the estimated activation threshold of this voltage-dependent current (ca. −52 mV) and its reversal potential (ca. −30 mV) (Maccaferri and McBain 1996; Svoboda

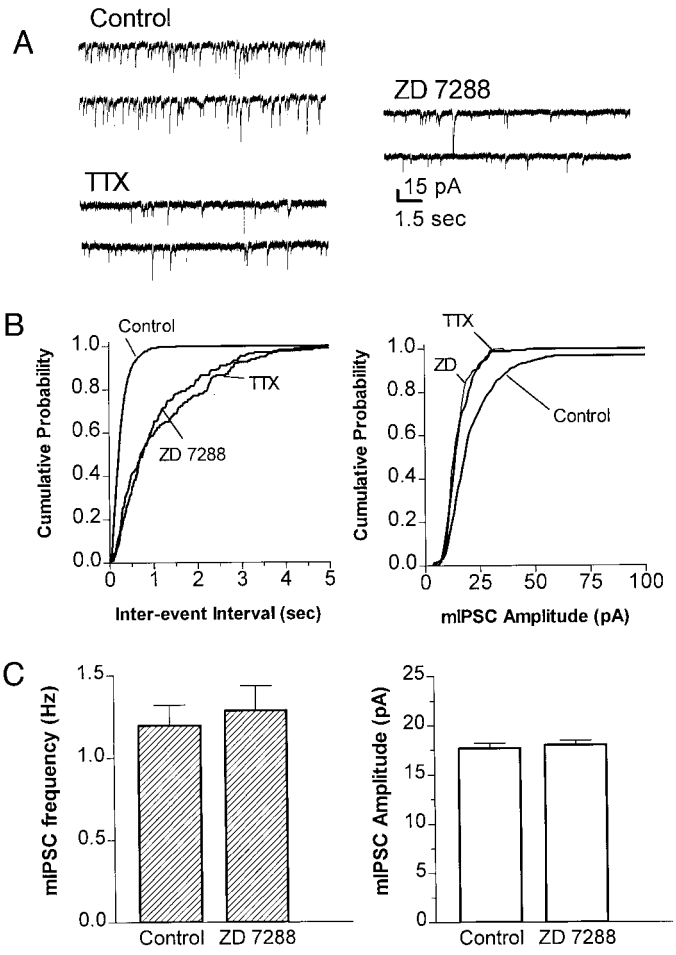

FIG. 6. Lack of effect of ZD 7288 (50 μM) on action potential-independent miniature IPSCs (mIPSCs) recorded in TTX in CA1 pyramidal neurons. *A*: traces illustrating the effects of TTX (0.5 μM) on sIPSCs and the subsequent effect of ZD 7288 after TTX application. Note the large reduction in sIPSC frequency and amplitude during TTX application and the lack of ZD 7288 effects on the mIPSCs in the presence of TTX. *B*: cumulative inter-event interval and amplitude distributions in the same CA1 pyramidal neuron shown in *A*. Note that TTX greatly lengthened the interval between the spontaneous currents (i.e., decreased frequency), and decreased their amplitude. Also note that the subsequent application of ZD 7288 did not cause further alterations in these parameters. *C*: group data ($n = 15$) illustrating the lack of effect of ZD 7288 on mIPSC frequency and amplitude in CA1 pyramidal neurons. All recordings were performed in the presence of ionotropic glutamate receptor antagonists.

and Lupica 1998) were positive to the RMP of these cells (ca. −61 mV), suggesting that I_h was partially active at rest. Second, both cesium and the selective blocker of I_h, ZD 7288 (BoSmith et al. 1993), resulted in a significant hyperpolarization of the resting interneuron membrane potential that was consistent with the blockade of this inward current. A final piece of data supporting the idea that I_h contributes to a significantly depolarized RMP was obtained from the small population of s.o. interneurons that do not express this current (i.e., <10%) (Svoboda and Lupica 1998). These neurons exhibited RMPs that were significantly more negative than those observed in the larger population of s.o. interneurons in which I_h was observed. This supports the hypothesis that I_h contributes to more depolarized RMPs and suggests that the absence of this current can serve as a predictor of the RMP. Collectively, these data suggest that the inward I_h is active at the RMP, thereby counterbalancing the tonically active outward currents (i.e., K^+ currents) that would shift the membrane potential toward more hyperpolarized levels if unopposed. At the present time, it is unclear why the membrane potential did not more closely approach the K^+ reversal potential (i.e., $E_k = -96$ mV) following blockade of I_h by ZD 7288. However, it is possible that additional inward currents are present in these neurons that were not blocked by ZD 7288 or cesium.

The results of the present study also suggest that the depolarizing influence of I_h is partly responsible for the continued release of GABA onto postsynaptic CA1 pyramidal neurons and thus the tonic inhibition of their activity. This is supported by data demonstrating that application of ZD 7288 caused a substantial decrease in the frequency of sIPSCs recorded in these cells. Previous studies have provided strong evidence that the majority of s- and mIPSCs recorded in hippocampal principal neurons arise from GABAergic interneurons, known as basket or perisomatic cells, that provide dense synaptic input to their somata (Miles et al. 1996; Soltesz et al. 1995). In the context of the present study, this suggests that at least some of these neurons express I_h channels that are active at the RMP and that this current contributed to the continued release of GABA and the tonic inhibition of CA1 pyramidal cells. We have previously demonstrated that >90% of the interneurons with somata located in s.o. of area CA1 express whole cell currents exhibiting characteristics consistent with I_h (Svoboda and Lupica 1998). However, we also found that only ~16% of these s.o. interneurons possessed axons that terminated in the CA1 pyramidal cell body layer (Svoboda et al. 1999). Whereas it is possible that these neurons were primarily responsible for the ZD 7288-sensitive sIPSCs observed in the present study, it is also possible that other groups of interneurons with somata located outside of s.o. that also express I_h may project to s. pyramidale. In fact, HCN mRNA expression (HCN1 and HCN2) that is likely associated with hippocampal interneurons has been described in s.o., s. pyramidale, s. radiatum, and s. lacunosum-moleculare in the CA1 region of the hippocampus (Santoro et al. 1997, 2000). Thus many different interneuron classes may express these hyperpolarization-activated cation channels, and these cells may also project to CA1 pyramidal neuron somata. Therefore we cannot exclude the possible contribution that these neurons may have made to the ZD 7288-sensitive sIPSC population in the present study.

The absence of effects of ZD 7288 on action potential-independent mIPSCs implies that the primary mechanism through which I_h alters tonic GABAergic inhibition is by decreasing the somatodendritic excitability of hippocampal interneurons. Furthermore, this suggests that hyperpolarization-activated ion channels, which have been immunohistochemically localized to inhibitory basket cell terminals in the hippocampus (Santoro et al. 1997), are probably not more directly involved in the neurotransmitter release process under basal conditions. These data are in contrast to those observed at the crayfish neuromuscular junction where I_h facilitated action potential-independent neurotransmitter release (Beaumont and Zucker 2000), although this was observed only when the magnitude of I_h was augmented via an increase in cAMP accumulation (Beaumont and Zucker 2000). In the present study, postsynaptic changes in GABA sensitivity or pyramidal neuron membrane time constants can be excluded as possible explanations for the effect of ZD 7288 on sIPSCs since mIPSC kinetics (i.e., decay time constants) were unaffected. Furthermore, I_h was blocked in CA1 pyramidal neurons by the addition of QX-314 to the pipette solution (Perkins and Wong 1995), eliminating postsynaptic blockade of I_h by ZD 7288 as an explanation for the modulation of sIPSCs. Based on these observations we hypothesize that blockade of I_h by ZD 7288 resulted in the hyperpolarization of interneurons, a decrease in action potential frequency, and a diminution of the amount of action potential-dependent GABA release onto the CA1 pyramidal neurons. In this respect these results are similar to those reported for cerebellar basket cells (Southan et al. 2000). However, it is not necessary to invoke the modulation of I_h channels in the nerve terminals as an explanation for the observed modulation by ZD 7288 because, as the present data indicate, the reduction in somatic excitability appears to be sufficient to explain the decrease in GABA release.

The hyperpolarization-activated current is modulated by neurotransmitters in the peripheral and central nervous systems. This includes the facilitation of I_h by monoamines such as norepinephrine and serotonin through a shift of the voltage dependence of the current to more depolarized levels (Bergles et al. 1996; Bobker and Williams 1989; Ingram and Williams 1994; Maccaferri and McBain 1996; McCormick and Pape 1990a; Pape and McCormick 1989). In addition, I_h is inhibited by μ-opioid receptors in peripheral neurons (Ingram and Williams 1994), by μ- and δ-opioid receptors in central neurons (Svoboda and Lupica 1998), and by adenosine in central neurons (Rainnie et al. 1994). Furthermore, adrenergic receptor activation facilitates (Bergles et al. 1996), whereas opioid receptor activation inhibits spontaneous GABA release in this preparation (Cohen et al. 1992; Lupica 1995). This has led to the hypothesis that the opioid-induced inhibition and the adrenergic receptor-induced enhancement of I_h would result in altered interneuron excitability and corresponding changes in GABA release from these cells (Svoboda and Lupica 1998). However, because both adrenergic and opioid receptors modulate additional ion channels (e.g., inwardly rectifying K^+ channels) (Svoboda and Lupica 1998) in these same neurons, it has been difficult to demonstrate this directly. Whereas the inhibitory effect of ZD 7288 on I_h is generally larger than that of the opioids, qualitatively and functionally the effects on synaptic transmission should be similar. Therefore the present results support the hypothesis that neuromodulators that either positively or negatively alter the contribution of I_h to the interneuron RMP will in turn predictably alter the amount of GABA released onto postsynaptic principal neurons. Thus

monoamines would be predicted to increase (e.g., Bergles et al. 1996) and opioids decrease (Svoboda and Lupica 1998) interneuron excitability and GABA release via this mechanism. In this way the modulation of I_h by neurotransmitter receptor activation and its altered contribution to the neuronal RMP may provide an additional mechanism through which principal neuron activity and output may be modified in the hippocampus and throughout the central and peripheral nervous systems.

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