Increased Basal Synaptic Inhibition of Hippocampal Area CA1 Pyramidal Neurons by an Antiepileptic Drug that Enhances I_H

Bi-Wen Peng*,[1], Jason A Justice[2], Kun Zhang[2], Xiao-hua He[1] and Russell M Sanchez*,[2]

[1]Department of Physiology, School of Medicine, Wuhan University, Wuhan, Hubei, China; [2]Department of Surgery, College of Medicine, Texas A&M Health Science Center, Neuroscience Institute, Scott & White Hospital, and Central Texas Veterans Health Care System, Temple, TX, USA

The hyperpolarization-activated cation current (I_H) regulates the electrical activity of many excitable cells, but its precise function varies across cell types. The antiepileptic drug lamotrigine (LTG) was recently shown to enhance I_H in hippocampal CA1 pyramidal neurons, showing a potential anticonvulsant mechanism, as I_H can dampen dendrito-somatic propagation of excitatory postsynaptic potentials in these cells. However, I_H is also expressed in many hippocampal interneurons that provide synaptic inhibition to CA1 pyramidal neurons, and thus, I_H modulation may indirectly regulate the inhibitory control of principal cells by direct modulation of interneuron activity. Whether I_H in hippocampal interneurons is sensitive to modulation by LTG, and the manner by which this may affect the synaptic inhibition of pyramidal cells has not been investigated. In this study, we examined the effects of LTG on I_H and spontaneous firing of area CA1 stratum oriens interneurons, as well as on spontaneous inhibitory postsynaptic currents in CA1 pyramidal neurons in immature rat brain slices. LTG ($100\,\mu M$) significantly increased I_H in the majority of interneurons, and depolarized interneurons from rest, promoting spontaneous firing. LTG also caused an increase in the frequency of spontaneous (but not miniature) IPSCs in pyramidal neurons without significantly altering amplitudes or rise and decay times. These data indicate that I_H in CA1 interneurons can be increased by LTG, similarly to I_H in pyramidal neurons, that I_H enhancement increases interneuron excitability, and that these effects are associated with increased basal synaptic inhibition of CA1 pyramidal neurons.

INTRODUCTION

The 'H-current' (I_H) is a depolarizing cation current in neurons and cardiac atrial muscle, which is activated by membrane hyperpolarization and does not inactivate during steady hyperpolarization (Kaupp and Seifert, 2001; Robinson and Siegelbaum, 2003; Baruscotti *et al*, 2005). I_H is mediated by channels that are formed from molecular subunits known as HCN1-4, a nomenclature that is derived from their gating by hyperpolarization and modulation by cyclic nucleotides (Clapham, 1998). The expression and subcellular distribution of HCN subunits in the brain varies across different regions and cell types (Monteggia *et al*, 2000; Bender *et al*, 2001; Notomi and Shigemoto, 2004), conferring a range of properties and specific functions on I_H

in the shaping of neuronal membrane and network behavior (Bal and McCormick, 1996; Luthi *et al*, 1998; Luthi and McCormick, 1998; Magee, 1998, 1999; Berger *et al*, 2001; Poolos *et al*, 2002).

The antiepileptic drug lamotrigine (LTG) can increase I_H in hippocampal CA1 pyramidal neurons, and enhancement of I_H in these cells has been shown to decrease the passive propagation of excitatory postsynaptic potentials (EPSPs) from distal dendrites to the soma (Poolos *et al*, 2002). This was explained by an increase in resting dendritic membrane conductance, and the consequent shunting of distal EPSPs was proposed as a possible anticonvulsant mechanism. Remarkably, however, because of its activation by hyperpolarization and slow deactivation, increased I_H can also promote rebound firing of CA1 pyramidal neurons under conditions of excessive synaptic inhibition, converting perisomatic inhibition into rebound excitation (Chen *et al*, 2001). Thus, I_H enhancement in pyramidal neurons can have varied functional outcomes that depend critically on the patterns of afferent synaptic activity.

In some inhibitory interneurons of the hippocampus, I_H depolarizes the resting membrane potential and promotes spontaneous firing, as I_H blockade was shown to decrease spontaneous firing in a subset of interneurons in hippocampal area CA1 stratum oriens (s.o.) (Maccaferri and

*Correspondence: Dr B-W Peng, Department of Physiology, School of Medicine, Wuhan University, Wuhan, Hubei, Donghu Rd186no.; Wuhan, Hubei 430071, China, Tel: +86 27 87331345 602, Fax: +86 27 68759991, E-mail: pengbiwen@whu.edu.cn or
Dr RM Sanchez, Department of Surgery, College of Medicine, Texas A&M Health Science Center, and Central Texas Veterans Health Care System, 1901 S. 1st St., Bldg. 205, Temple, TX 76504, USA, Tel: +1 254 743 0544; Fax: +1 254 743 0555, E-mail: rsanchez@medicine.tamhsc.edu

McBain, 1996; Lupica *et al*, 2001). This decrease in interneuron excitability was further associated with a concomitant decrease in the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in CA1 pyramidal neurons (Lupica *et al*, 2001). These findings indicated that I_H in CA1 inhibitory interneurons promotes a basal level of synaptic inhibition of CA1 pyramidal cells by promoting the spontaneous firing of afferent interneurons. Thus, compounds that enhance I_H could exert complex functional effects on CA1 pyramidal neuron excitability if they also increase I_H in presynaptic inhibitory interneurons to promote a higher frequency of spontaneous IPSCs. It is noteworthy that the pattern of HCN subunit expression differs between pyramidal and non-pyramidal neurons in area CA1 (Bender *et al*, 2001; Vasilyev and Barish, 2002), and whether I_H in hippocampal interneurons is sensitive to modulation by LTG has not been investigated. Therefore, in this study, we investigated whether a compound that increases I_H in CA1 pyramidal neurons similarly increases I_H in CA1 interneurons, and if so, whether this increases spontaneous interneuron firing, with a concomitant increase in sIPSC frequency in CA1 pyramidal neurons.

MATERIALS AND METHODS

Animals

Male Long–Evans rat pups (Charles River), aged postnatal day 10–13, were used for these experiments. Litters were housed with their dam on a 12-h light–dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the NIH guidelines on the ethical use of experimental animals.

Hippocampal Slice Preparation

Rat pups were killed by decapitation under isoflurane anesthesia. The brains were removed and immediately placed in ice-cold oxygenated artificial cerebrospinal fluid (ACSF). A transverse razor cut was made anterior to the cerebellum and perpendicular to the midbrain, and the cut end was glued to the stage of a vibratome (Leica) for slicing. Slices of 370-μm thickness were cut in cold, continuously oxygenated ACSF, and then incubated for at least 1 h in a custom-made holding chamber filled with continuously oxygenated ACSF at room temperature.

Electrophysiological Recordings

Slices were transferred into a submersion chamber (Warner Instruments) that was superfused continuously with ACSF at room temperature for recordings. Whole-cell patch-clamp recordings were obtained from CA1 pyramidal neurons and from non-pyramidal neurons in area CA1 s.o. under visual guidance using infrared differential interference contrast microscopy (Zeiss Axioskop FS2 w/Dage-MTI IR-1000 CCD camera). Recorded neurons were presumed to be inhibitory interneurons on the basis of their non-pyramidal morphology and on their location outside the pyramidal cell layer. Intracellular labeling of a subset of recorded neurons confirmed that they were not pyramidal neurons (see Figure 1). All recorded pyramidal neurons were located within the stratum pyramidale, had soma diameters of 40–50 μm, and apical dendritic trunks extending perpendicular to the stratum pyramidale into the stratum radiatum.

Voltage-clamp recordings were obtained using a Multi-clamp 700A amplifier and digitized using Digidata 1322A (Molecular Devices) for acquisition to a computer. Voltage-clamp protocols were generated, and data were collected for acquisition to a computer using Clampex (PClamp 9.0, Molecular Devices). Both input resistance and series resistance were monitored intermittently throughout the experiments by applying 5 or 10 mV hyperpolarizing voltage steps from a holding potential of −40 or −70 mV (depending on the experimental protocol being used). Initial series resistances were estimated to be < 20 Mega-ohms, and data were discarded if series resistance changed by more than 20%. Data were analyzed off-line using Clampfit (Molecular Devices) and Mini-Analysis (Synaptosoft) on a Windows-based computer, as well as using AxoGraph 4.9 (Molecular Devices) and Igor Pro Carbon (Wavemetrics) on a Macintosh computer.

I_H was activated under voltage clamp by hyperpolarizing voltage steps from a holding potential of −40 mV with 1 μM tetrodotoxin in the bath. A subset of cells was recorded with 10 mM tetraethylammonium (TEA), 5 mM 4-aminopyridine (4-AP), and 200 μM $CdCl_2$ additionally included in the bath to block voltage-dependent potassium and calcium currents. Steady-state currents were determined by subtracting the initial current after the capacitance transient (leak current) from the mean steady current during the last 10–20 ms of the hyperpolarizing voltage command. Inward tail currents were measured at −70 mV (see Figure 2) and were normalized to the maximum tail current after an activation step to −130 mV. sIPSCs and miniature IPSCs (mIPSCs) were recorded at a holding potential of −70 mV with glutamate receptors blocked by NBQX (20 μM) and APV (50 μM). mIPSCs were isolated by adding 1 μM tetradotoxin to the bath. For statistical analyses, IPSCs (200–300 events for sIPSCs; 60–100 events for mIPSCs) recorded immediately before drug application and those beginning 10–12 min after the initiation of drug application were compared. For off-line IPSC analyses, sIPSC and mIPSCs were detected using Mini-Analysis (Synaptosoft), and pre- and post-drug statistical comparisons of inter-event intervals, amplitudes, as well as rise and decay time constants (τ estimated from single exponential fits) were performed using the Kolmogorov–Smirnov two-sample test in Mini-Analysis.

Solutions

The ACSF consisted of (in mM): 126 NaCl, 3.3 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 D-glucose. The internal patch solution for interneuron recordings consisted of (in mM): 145 K-gluconate, 10 HEPES, 1 EGTA, 10 KCl, 0.1 $CaCl_2$, 0.2 NaATP and 2 MgATP (pH adjusted to 7.3; 280–290 mOsm). Biocytin (0.1%) was freshly added to the patch solution for interneuron recordings to intracellularly label cells for morphological identification. For recording synaptic events from pyramidal neurons, the internal patch solution consisted of (in mM): 129 CsCl, 10 HEPES, 10 EGTA, 2 $MgCl_2$, 0.3
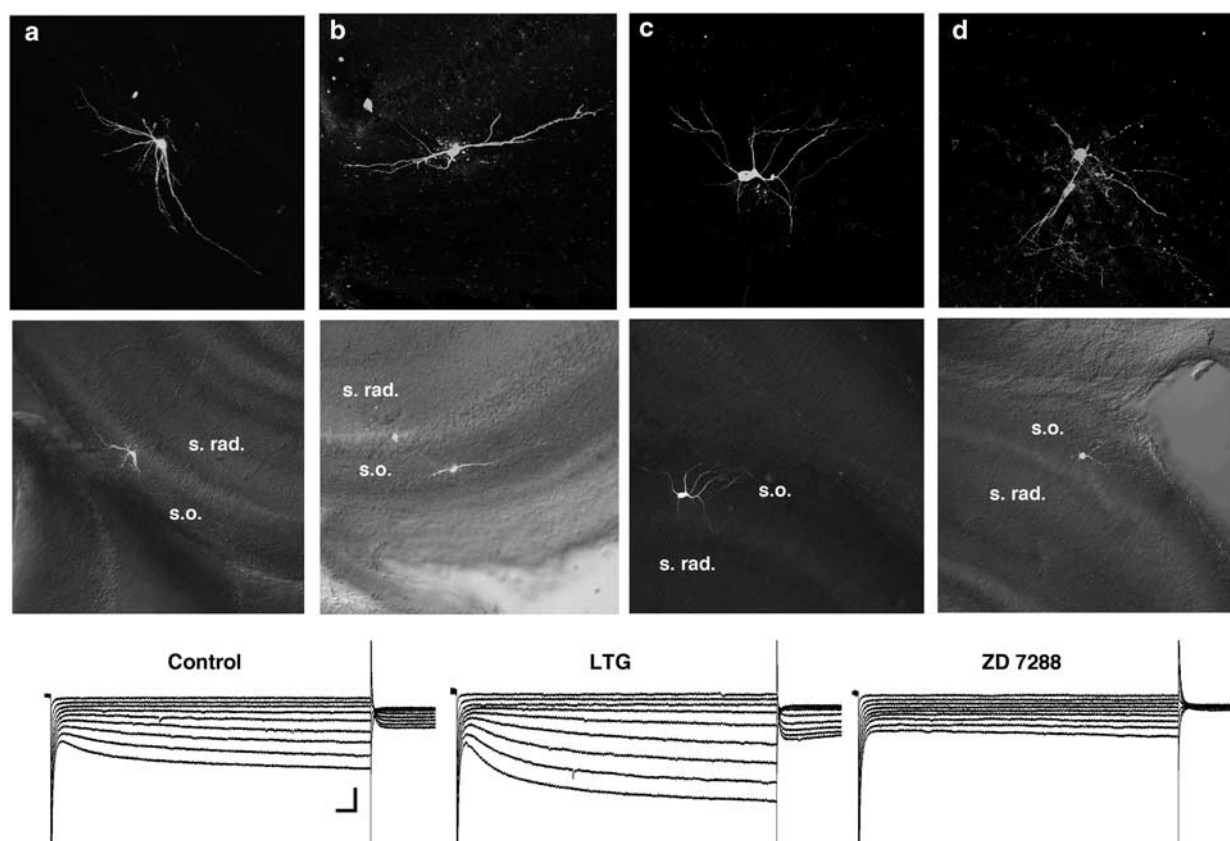
Figure 1 LTG increased I_H in CA1 interneurons. (Top; a–d): four different interneurons that were intracellularly labeled during recording are shown. Each panel below showed the same cell at lower magnification superimposed on the bright-field DIC image to show the cell location. The cells in panels a and b each exhibited I_H that was increased by LTG (data below are from the cell in panel b), whereas the cells in panels c and d exhibited no apparent baseline I_H. It must be noted that the two cells that exhibited I_H have dendritic fields that are oriented horizontally and appear to be entirely within the s.o., in contrast to the cells in panels c and d. (s.o. = stratum oriens; s.rad. = stratum radiatum;). Lower panels: raw current responses under voltage clamp for representative CA1 s.o. non-pyramidal neurons to hyperpolarizing voltage steps (−45 to −125 mV in 10-mV increments) from a holding potential of −40 mV are shown. The left-most panels (Control) show the slowly activating, non-inactivating inward current characteristic of I_H. The middle panels show the responses of the same cell in the presence of LTG, and an apparent increase in I_H can be seen. The right-most panels illustrate inhibition of the slow inward current by the I_H blocker ZD 7288, confirming that the inward current was I_H (scale bar: 200 ms, 100 pA).

NaATP, and 4 MgATP (pH adjusted to 7.3; 280–290 mOsm). All drugs were applied by bath superfusion. NBQX, APV, TEA, and 4-AP were dissolved in distilled water at high concentration, aliquoted and frozen, and then thawed as required and diluted $100 \times$ in the ACSF. LTG was freshly dissolved at the final concentration directly in ACSF. NBQX, APV, TEA, and 4-AP were obtained from Sigma-Aldrich, and LTG was a gift from Dr Jose Cavazos, Department of Medicine (Neurology), UTHSCSA (University of Texas Health Science Center at San Antonio).

Histological Recovery of Biocytin-Filled Cells

Immediately after electrophysiological recordings, slices with biocytin-filled cells were fixed by submersion in cold paraformaldehyde (PFA; 4% in phosphate buffer) in a small petri dish, and flattened by laying a coverslip on the slice. Slices were kept overnight in PFA at 4°C. The next day, PFA was replaced with 30% sucrose in phosphate buffer and slices were stored overnight to 1 week at 4°C. The slices were washed thrice (3 min each) at room temperature, and then incubated in PBS that contained a 1 : 250 dilution of

Alexa Fluo 488-conjugated streptavidin (Molecular Probes), 3% normal goat serum, and 0.25% Triton-X for 4 h at 4°C. The slices were then washed again thrice (3 min each) in PBS at room temperature, and mounted on concavity slides and coverslipped with the Vectashield (Vector Labs) mounting medium. Labeled cells were visualized on an Olympus FV-500 Confocal Laser Scanning Microscope in the UTHSCSA Core Optical Imaging Facility.

RESULTS

Effects of LTG on I_H in CA1 s.o. Interneurons

Given the diversity of HCN channels and their nonuniform expression in the brain (Monteggia *et al*, 2000; Bender *et al*, 2001; Notomi and Shigemoto, 2004), it is possible that the sensitivity of I_H to pharmacological modulation may vary across cell types because of differences in the expressed HCN isoforms. Therefore, we first examined whether LTG could modulate I_H in area CA1 interneurons. Figure 1 (lower part) shows representative raw traces of the slowly developing non-inactivating inward current presumed to be
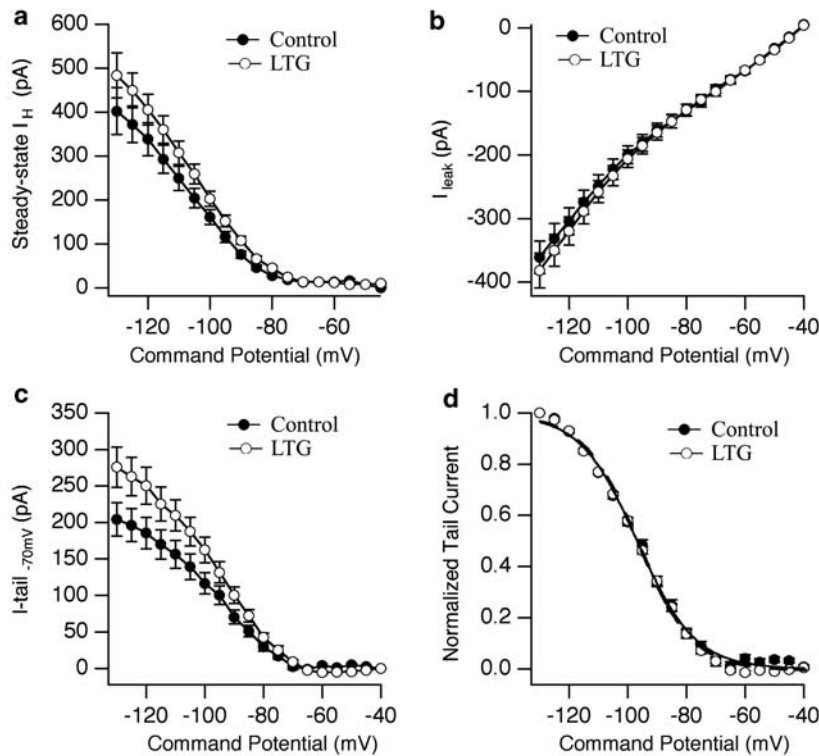
Figure 2 LTG increased I_H in CA1 interneurons by increasing maximal conductance. (a) Pooled leak-subtracted steady-state I_H showed a significant increase in the maximum current in response to LTG application. (b) Pooled leakage currents measured at the end of the initial capacitance transient and before development of the slow inward current showed no effect of LTG. This indicated that LTG specifically increased the slower inward I_H. (c) Raw tail currents again showed an increase by LTG, indicating increased maximal conductance. (d) Tail currents normalized to the maximum tail current after a command step to −130 mV in each condition showed that LTG did not alter the voltage dependence of I_H activation. The solid and dashed lines are Boltzmann's fits to the pooled mean normalized currents.

I_H in non-pyramidal neurons. As illustrated by the photomicrographs of recorded cells in Figure 1a–d, intracellular labeling confirmed that our recordings were from non-pyramidal interneurons of the s.o. Within this population, an apparent baseline I_H was observed in 89% (61/68) of recorded cells. Panels a and b show two cells that exhibited I_H (the traces shown just below are from the cell in panel b), whereas panels c and d show two cells that did not exhibit I_H. Similar to a previous report (Maccaferri and McBain, 1996), the majority (14/15) of recovered cells that exhibited I_H had horizontally oriented dendritic fields that were confined to the s.o., whereas all three recovered cells that did not exhibit I_H had dendrites that extended into the stratum radiatum. As can be seen in the raw traces in Figure 1, application of 100 μM LTG caused an apparent increase in I_H within 5–7 min (data shown were obtained 10 min after the initiation of LTG superfusion), and this effect of LTG was observed in 75% (21/28) of interneurons that expressed I_H. As shown in the far right panels, subsequent application of the specific I_H blocker ZD 7288 (50 μM) completely blocked the slowly activating inward current, further supporting its identification as I_H.

To better isolate and examine the effects of LTG on I_H in CA1 s.o. interneurons more quantitatively, some cells were recorded with voltage-gated potassium and calcium channels blocked by TEA (10 mM), 4-AP (5 mM), and $CdCl_2$ (200 μM). Figure 2 showed pooled data for steady-state and tail currents acquired under these conditions.

As shown in Figure 2a, the steady-state I_H measured in this manner was significantly increased by LTG ($p < 0.0001$, two-way ANOVA with matching, $n = 8$), whereas the leak current was not altered ($p = 0.68$, Figure 2b). Tail currents were also significantly increased by LTG ($p < 0.0001$, Figure 2c). Examination of normalized tail currents (see 'Materials and methods' section) indicated no changes in the voltage dependence of I_H activation by LTG (Figure 2d). These data indicated that LTG could significantly increase I_H in a subset of CA1 s.o. interneurons by increasing the maximum macroscopic conductance.

Effect of LTG on Interneuron Resting Activity

Given previous reports that I_H blockade decreased the spontaneous firing of CA1 s.o. interneurons (Maccaferri and McBain, 1996; Lupica et al, 2001), we next investigated whether an agent that increases I_H in these cells could increase their spontaneous firing. Both resting potentials and spontaneous action potentials were examined in CA1 s.o. interneurons that showed an apparent I_H under current clamp (without tetradotoxin) before and during the application of LTG. As shown for a representative cell in Figure 3, LTG induced both an apparent increase in the depolarizing 'sag' in response to hyperpolarizing current injection and depolarization of the resting membrane potential and increases in spontaneous firing in the majority (13/16) of cells (Figure 3b). The mean resting
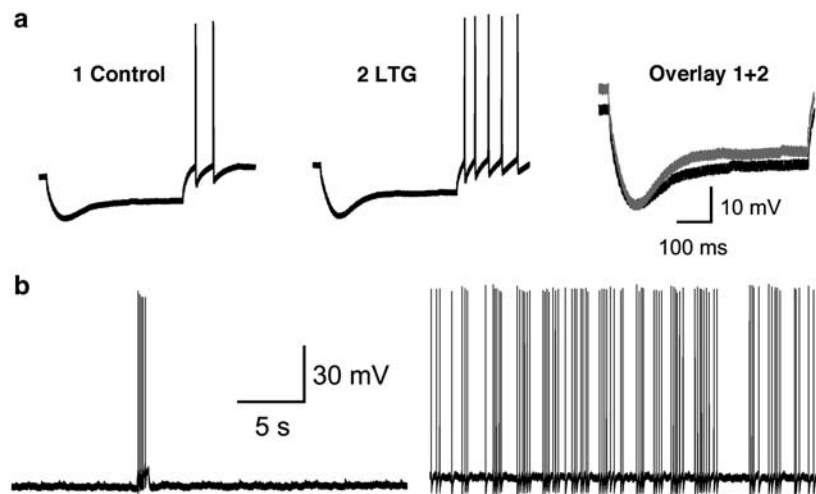
Figure 3 LTG depolarized CA1 stratum oriens interneurons. Current-clamp traces for two cells before and during LTG application are shown. (a) Voltage responses to hyperpolarizing current injection showed a depolarizing 'sag' that is characteristic of I_H. In the presence of LTG, current injection to reach the same level of hyperpolarization (a2) as in control (a1) showed that the depolarizing sag was increased. (b) Spontaneous activity of the same neuron under the two conditions. Before LTG, this cell showed an occasional burst of action potentials, but in the presence of LTG, the cell fired continued brief action potential bursts. Statistical comparisons of pooled data indicated that LTG significantly depolarized the resting membrane potential (see text) (scale bars: (top) 100 ms, 10 mV; (bottom) 5 s, 30 mV).

membrane potential was significantly depolarized from -61.6 ± 1.8 mV before LTG to -57.1 ± 2.9 mV during LTG application ($p = 0.03$, paired t-test, $n = 16$). The mean action potential frequency in cells that fired spontaneously before LTG application increased by $789 \pm 177\%$ after LTG ($n = 6$, $p < 0.05$). These data indicated that augmented I_H by LTG could promote spontaneous firing in a subset of CA1 s.o. interneurons, consistent with the previously proposed role of I_H in these cells.

### Effects of LTG on sIPSC Frequency in Pyramidal Neurons

We next investigated whether the LTG effects on interneuron spontaneous activity were associated with changes in the frequency of sIPSCs in CA1 pyramidal neurons. LTG was observed to increase sIPSC frequency significantly in 100% (7/7; $p < 0.001$, Kolmogorov–Smirnov test) of cells (Figure 4) within 10 min of application with no changes in amplitudes or in rise and decay times (see Table 1). Table 1 shows mean ($\pm$ SEM) sIPSC parameters under each condition. Paired t-tests of pre- and post-drug means for sIPSC parameters indicated no significant changes in sIPSC amplitudes ($p = 0.71$), rise times ($p = 0.75$), or decay times ($p = 0.57$), and a significant increase in frequency ($p = 0.046$). In addition, a previous application of ZD 7288 (50 μM) significantly decreased sIPSC frequency compared with baseline ($p < 0.0001$; $n = 3$) and prevented any subsequent drug-induced changes in sIPSC parameters (Figure 5). These data indicated that the increase in sIPSC frequency most likely resulted from increased spontaneous firing of interneurons that directly provide synaptic inhibition of CA1 pyramidal neurons, consistent with increased interneuron excitability by the augmentation of I_H.

LTG did not Alter mIPSCs

Finally, to determine whether LTG may have altered presynaptic GABA release directly at axon terminals, its effects on mIPSCs recorded from CA1 pyramidal neurons were investigated. As illustrated for representative cells in Figure 6, LTG exerted no effect on mIPSC frequency or amplitudes, as determined within individual cells by Kolmogorov–Smirnov analysis ($n = 60$–100 events per condition). Mean mIPSC amplitudes were 36 ± 3.9 pA before LTG and 36.6 ± 3.7 pA during LTG ($p = 0.7$, paired t-test, $n = 6$ cells), and mIPSC frequencies were 0.19 ± 0.05 Hz (control) and 0.17 ± 0.05 Hz (LTG; $p = 0.44$, paired t-test, $n = 6$). These data indicated that LTG altered only the action potential-dependent IPSCs, and were consistent with the hypothesis that the increased sIPSC frequency was due to increased spontaneous firing of afferent inhibitory interneurons.

DISCUSSION

Our data showed that LTG increased I_H in a subset of non-pyramidal neurons in hippocampal area CA1 s.o.. LTG also depolarized the resting membrane potential and/or increased spontaneous firing in the majority of non-pyramidal neurons studied, and this was associated with an increase in the frequency of sIPSCs recorded from CA1 pyramidal neurons. Although we cannot determine from our data the precise identity and synaptic targets of the cells that mediated the increased sIPSC frequency by LTG, the absence of changes in sIPSC amplitudes or rise and decay times, or in mIPSC parameters is consistent with an increase in spontaneous firing of afferent inhibitory neurons as the underlying mechanism. In addition, the effects of LTG on both I_H and sIPSCs were prevented
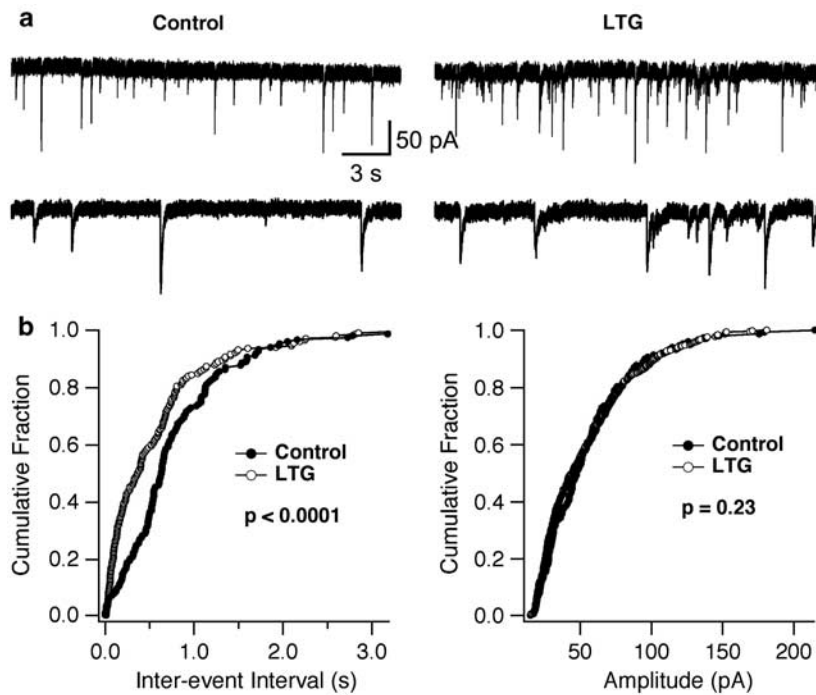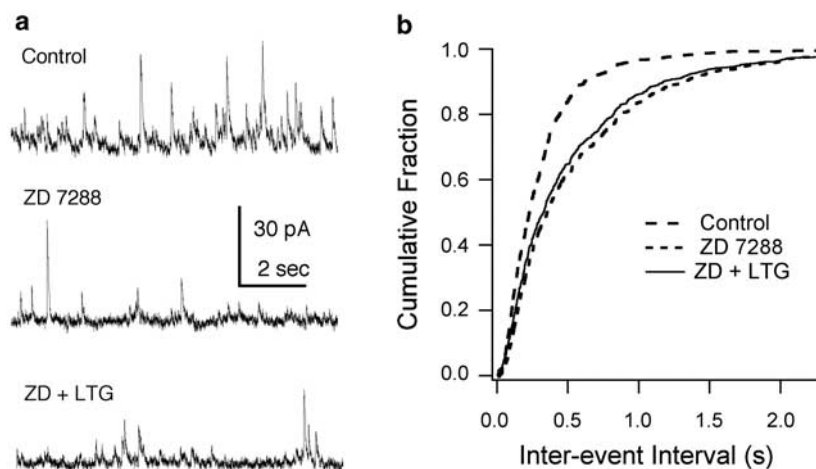
Figure 4 LTG increased sIPSC frequency in CA1 pyramidal neurons. (a) sIPSCs recorded from a CA1 pyramidal neuron before and during LTG application showed an apparent increase in frequency. (b) Cumulative histograms showed a significant decrease in sIPSC inter-event interval after LTG application with no significant change in sIPSC amplitudes (Kolmogorov–Smirnov two-sample test). Statistical comparisons of pooled data showed that only sIPSC frequency was altered by LTG (see text and Table 1) (scale bars for panel a: (top traces) 3 s, 50 pA; (bottom traces), 0.6 s, 50 pA).

Table 1 Mean sIPSC Parameters Under Each Condition

Condition	Amplitudes (pA)	Frequency (Hz)	Rise times (ms)	Decay τ (ms)
Control	37.8 ± 2.6	2.64 ± 0.6	1.13 ± 0.07	30.6 ± 1.6
LTG	39.5 ± 5.0	4.69 ± 1.66	1.15 ± 0.1	32.2 ± 3.1

Mean and SE are shown for sIPSC amplitudes, frequency, rise times, and decay time constants (τ) for those cells that exhibited a significant increase in sIPSC frequency. No significant effects of LTG on parameters other than frequency were observed.

Figure 5 I_H blockade decreased sIPSC frequency in pyramidal neurons and prevented the LTG-induced frequency increase. (a) Raw traces showed sIPSCs recorded at 0 mV from a pyramidal neuron in control ACSF, during application of ZD 7288, and during subsequent application of ZD + LTG. (b) Cumulative histograms showed that ZD increased sIPSC inter-event interval (decreased frequency) and prevented the effect of LTG.
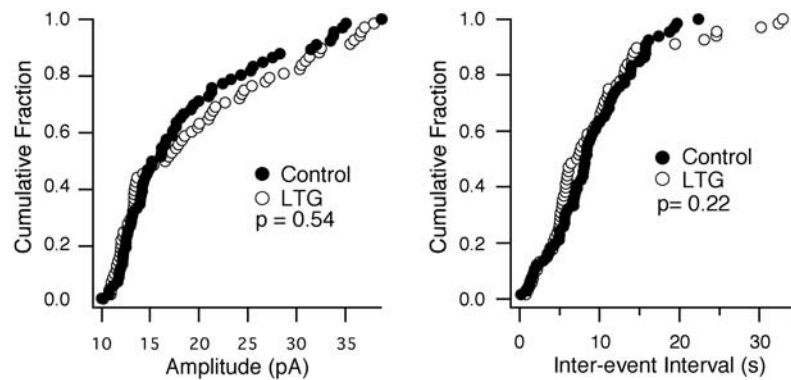
Figure 6 LTG did not alter mIPSCs in CA1 pyramidal neurons. Cumulative histograms for two recorded pyramidal neurons showed no significant changes in mIPSC amplitudes or inter-event intervals or amplitudes by LTG. Statistical comparisons of pooled data confirmed no significant effect of LTG on mIPSC parameters (see text).

or reversed by the I_H inhibitor, ZD 7288. Taken together, these data indicated that an indirect effect on CA1 pyramidal neurons of global I_H enhancement by LTG is to increase their basal synaptic inhibition by increasing the spontaneous firing of presynaptic inhibitory neurons.

It is worth noting that non-pyramidal neurons in hippocampal area CA1 commonly designated as 'interneurons' represent heterogeneous populations for which no unifying classification scheme exists. Nonetheless, sub-populations have been described on the basis of anatomical location and cell morphology, axonal/synaptic projections, expression of neuropeptides or calcium-binding proteins, electrophysiologically defined intrinsic and synaptic properties, or combinations of these (Maccaferri and Lacaille (2003). This study targeted non-pyramidal neurons in the s.o. to be consistent with previous studies of I_H regulation of interneuron excitability (Maccaferri and McBain, 1996; Lupica et al, 2001), and used cell morphology to provide some information as to the likely identity of recorded cells. Of histologically recovered cells that expressed I_H, nearly all (14 out of 15) exhibited horizontally oriented dendritic fields that were confined to the s.o., which is characteristic of cells that have been categorized as oriens-lacunosum moleculare or O-LM neurons. In contrast, the three recovered cells that showed no discernible I_H exhibited dendritic arbors that appeared typical of stellate (Figure 1c) or basket cells (Figure 1d) and extended across the pyramidal cell layer into the stratum radiatum. Putative O-LM neurons could not be conclusively identified because the axonal projections that distinguish this sub-population from other horizontally oriented cells could not be resolved using our intercellular labeling technique. Nonetheless, these data would seem to be consistent with previous studies that have shown a prominent I_H in O-LM neurons (Maccaferri and McBain, 1996; Ali and Thomson, 1998; Svoboda and Lupica, 1998).

Mechanisms of I_H Modulation

LTG was reported to enhance I_H in pyramidal neurons through a positive shift in the voltage dependence of I_H activation, thus increasing the channel open probability at a

given physiological membrane potential (Poolos et al, 2002). In contrast, our data indicated that LTG increased the maximum macroscopic HCN channel conductance in interneurons without a change in the voltage dependence of activation. Differences in the HCN isoforms expressed or in cell-specific signaling could have contributed to the observed difference in LTG effects, as well as to the inter-cell variability in drug responses in our study, particularly given the known diversity of many properties of hippocampal interneurons (for a review, see Maccaferri and Lacaille, 2003). For example, in postnatal day 11 rat, CA1 pyramidal neurons and stratum radiatum interneurons exhibit high expression of HCN1 mRNA, whereas CA1 s.o. interneurons predominantly express HCN2 with only rare detection of HCN1 (Bender et al, 2001). Chen et al (2005) showed that the volatile anesthetic, halothane, negatively modulates I_H by shifting the voltage dependence of activation in HEK 293 cells that express HCN1, but by decreasing the maximum macroscopic conductance in cells that express HCN2. At present, subunit-specific differences in the effects of LTG remain speculative, as the precise mechanisms by which LTG modulates HCN channels are yet unknown.

The possibility that differences in the LTG effect on non-pyramidal vs pyramidal neurons resulted from differences in recording modes also cannot be excluded, as Poolos et al (2002) used cell-attached recordings, whereas this study used whole-cell recordings, which alter the intracellular milieu. The findings by Chen et al (2005) of HCN subunit-specific effects of halothane on I_H also used whole-cell recordings. However, when a saturating concentration of cAMP was included in the recording pipette, halothane shifted the voltage dependence of HCN2 channels with little effect on macroscopic conductance, similar to its effects on HCN1 channels. Thus, if the observed mechanism of I_H modulation by LTG also depended on baseline conditions, the difference in LTG effect on pyramidal vs non-pyramidal neurons could have been due at least in part to the difference in recording modes between studies. Such dependence would also suggest that the modulation of I_H by LTG is dynamic and highly dependent on the recent activity of the cell.

Potential Role of Dendritic Shunting

Despite variability in the observed drug effects across individual interneurons, the effect of LTG on populations of interneuron, as indicated by increased sIPSC frequency in CA1 pyramidal neurons, was observed in every pyramidal cell tested. This is particularly interesting, given the previous demonstration that increased I_H by LTG administration can dampen distal dendritic EPSCs in CA1 pyramidal neurons through electrical shunting (Poolos *et al*, 2002). Different interneuron types innervate specific cellular compartments (Maccaferri *et al*, 2000), and in particular, oriens-lacunosum-moleculare (OL-M) neurons (the dendritic fields of which reside within the s.o.) primarily innervate the most distal pyramidal cell dendrites (Sik *et al*, 1995; Maccaferri *et al*, 2000). Thus, increased I_H would be expected to dampen passive dendro-somatic transmission of these inhibitory synaptic currents in addition to excitatory currents. This study aimed at identifying LTG effects on afferent inhibitory neurons using postsynaptic IPSCs as a measure of the modulation of these afferents. For this reason, intracellular Cs + was used as a channel blocker to prevent the potential confound of experimentally induced changes in shunting conductances (including G_H). Therefore, this study could not address the potential effects of altered electrotonic filtering by I_H modulators. Although increasing excitability in a subset of interneurons by global I_H enhancement would seem sufficient to significantly increase the basal synaptic inhibition of CA1 pyramidal neurons, further work will be necessary to determine the extent to which dendritic filtering of IPSCs could oppose the effects of increased frequency of synaptic inhibition.

Global I_H Modulation as an Anticonvulsant Strategy

Recent evidence indicated that I_H can be pathologically altered in principal limbic neurons in models of seizure that induce epileptogenesis or chronically increased seizure susceptibility (Shah *et al*, 2004; Poolos *et al*, 2002; Zhang *et al*, 2006). I_H in entorhinal cortical layer III neurons was shown to be decreased for at least 1 week after kainate-induced status epilepticus in adult rats, and this was associated with enhanced passive EPSP propagation and increased synaptic excitability of these cells during this epileptogenic 'silent period' (Shah *et al*, 2004). A similar acute and persistent decrease in I_H was observed in hippocampal CA1 pyramidal neurons after seizures induced by global hypoxia in P10 rats (Zhang *et al*, 2006). Combined with the observation that increased I_H by LTG resulted in decreased dendritic synaptic excitability of CA1 pyramidal neurons (Poolos *et al*, 2002), these findings suggested that pathological I_H downmodulation is pro-epileptogenic and that LTG may exert an antiepileptogenic effect by increasing I_H in pyramidal cells. However, it is worth noting that CA1 pyramidal neurons in the immature rat brain after hyperthermia-induced seizures at P10-11 exhibited functionally increased I_H; yet, this was also found to enhance pyramidal neuron excitability by converting increased basal synaptic inhibition into rebound excitation mediated by I_H tail currents (Chen *et al*, 1999, 2001). This suggested that global I_H enhancement by LTG could promote the conversion of synaptic inhibition into excitation to promote network excitability and seizures under conditions in which IPSC frequency is increased beyond a critical threshold. Given the different changes to I_H and basal synaptic inhibition reported in models that each result in chronically increased seizure susceptibility (Dube and Baram, 2006; Dudek *et al*, 2006; Sanchez and Jensen, 2006), it is conceivable that pharmacological I_H enhancement may be antiepileptogenic in some settings, and that I_H inhibition may be antiepileptogenic in others. Pharmacological targeting of I_H upmodulation or downmodulation to specific cell types or HCN subunits may ultimately be a more efficacious antiepileptogenic and/or anticonvulsant strategy.

DISCLOSURE

The authors declare no conflict of interest.

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