Lidocaine Blocks the Hyperpolarization-activated Mixed Cation Current, $I_h$, in Rat Thalamocortical Neurons

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ABSTRACT

Background: The mechanisms that underlie the supraspinal central nervous system effects of systemic lidocaine are poorly understood and not solely explained by Na$^+$ channel blockade. Among other potential targets is the hyperpolarization-activated cation current, $I_h$, which is blocked by lidocaine in peripheral neurons. $I_h$ is highly expressed in the thalamus, a brain area previously implicated in lidocaine’s systemic effects. The authors tested the hypothesis that lidocaine blocks $I_h$ in rat thalamocortical neurons.

Methods: The authors conducted whole cell voltage- and current-clamp recordings in ventrobasal thalamocortical neurons in rat brain slices in vitro. Drugs were bath-applied. Data were analyzed with Student t tests and ANOVA as appropriate; $\alpha = 0.05$.

Results: Lidocaine voltage-independently blocked $I_h$, with high efficacy and a half-maximal inhibitory concentration (IC$_{50}$) of 72 mM. Lidocaine did not affect $I_h$ activation kinetics but delayed deactivation. The $I_h$ inhibition was accompanied by an increase in input resistance and membrane hyperpolarization (maximum, 8 mV). Lidocaine increased the latency of rebound low-threshold Ca$^{2+}$ spike bursts and reduced the number of action potentials in bursts. At depolarized potentials associated with the relay firing mode ($> -60$ mV), lidocaine at 600 $\mu$M concurrently inhibited a K$^+$ conductance, resulting in depolarization ($7–10$ mV) and an increase in excitability mediated by Na$^+$-independent, high-threshold spikes.

Conclusions: Lidocaine concentration-dependently inhibited $I_h$ in thalamocortical neurons in vitro, with high efficacy and a potency similar to Na$^+$ channel blockade. This effect would reduce the neurons’ ability to produce intrinsic burst firing and δ rhythms and thereby contribute to the alterations in oscillatory cerebral activity produced by systemic lidocaine in vivo.

What We Already Know about This Topic

• The mechanisms underlying the supraspinal central nervous system effects of lidocaine are poorly understood and not solely explained by Na$^+$ channel blockade, but may involve the hyperpolarization-activated cation current, $I_h$, which is blocked by lidocaine in peripheral neurons

What This Article Tells Us That Is New

• Lidocaine concentration-dependently inhibited $I_h$ in rat thalamocortical neurons in vitro, with high efficacy and a potency similar to Na$^+$ channel blockade
• A resultant reduction of intrinsic burst firing and δ rhythms may contribute to the alterations in oscillatory cerebral activity produced by systemic lidocaine in vivo
which is blocked by lidocaine in peripheral sensory neurons,\(^1\) \(I_h\) predominantly its underlying channel isoform, HCN2,\(^12\) is highly expressed in the thalamus,\(^13–15\) a brain area that plays an important role in the generation of the different physiologic conscious states and associated cerebral rhythms; in drug-induced sedation, anesthesia, and analgesia; and in epileptogenesis.\(^16–19\) In mammals, lidocaine at subconvulsive doses has long been known to produce slow-wave electroencephalographic rhythmic activity and “spindling” associated with sedation and reduced responsiveness to noxious stimuli;\(^20–23\) implicating the thalamus as a site of action. More recently, \textit{in vitro}\(^24,25\) and human \textit{in vivo}\(^26\) reports have focused on lidocaine’s actions in the ventrobasal thalamus, the main supraspinal relay station for somatosensory and nociceptive signals.\(^27\) However, lidocaine’s effects on \(I_h\) in ventrobasal thalamocortical neurons are unknown.

\(I_h\), whose activation produces a depolarizing nonactivating inward current,\(^12\) is crucial for controlling excitability in thalamocortical neurons in multiple ways. First, it contributes to the setting of the resting membrane potential (RMP), as a significant fraction of \(I_h\) channels is active near rest.\(^12,28–30\) Second, because of its leak and negative-feedback properties, \(I_h\) operates as a “voltage-clamp,” passively shunting incoming impulses and actively opposing hyperpolarization and depolarization.\(^12,31,32\) As a result, \(I_h\) is critical for determining the distinct voltage-dependent firing mode of these neurons. At depolarized potentials positive to approximately \(-60\) mV, they exhibit a “relax” or “tonic” mode that is associated with vigilance and wakefulness \textit{in vivo} and characterized by tonic repetitive firing of singleton Na\(^+\)-dependent action potentials.\(^16,33\) At hyperpolarized potentials, neurons switch to the “oscillatory” or “burst” mode that occurs in states of slow-wave electroencephalographic activity (e.g., nonrapid eye movement sleep) and features action potential bursts mediated by the low-threshold Ca\(^{2+}\) current, \(I_T.\)\(^12,16,33\) Third, through interaction with \(I_T\) to generate rhythmic burst firing, \(I_h\) serves as a pacemaker current and is central to the generation of slow intrinsic neuronal\(^28\) and network oscillations in the thalamocortical system during nonrapid eye movement sleep and drowsiness.\(^34–36\)

Here, we tested the hypothesis that lidocaine blocks \(I_h\) in rat ventrobasal thalamocortical neurons \textit{in vitro} and explored the functional consequences of \(I_h\) blockade by lidocaine in these neurons.

### Materials and Methods

#### Preparation of Brain Slices

Ethics approval for all animal experiments was obtained from the Committee on Animal Care of The University of British Columbia (Vancouver, British Columbia, Canada). Wistar rats of postnatal age P13–P16 were deeply anesthetized with isoflurane (Abbott Laboratories, Montreal, Canada) and decapitated. The cerebrum was rapidly removed and placed in oxygenated (5% CO\(_2\)/95% O\(_2\), cold (1–4°C), artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 124; KCl, 2.5; NaH\(_2\)PO\(_4\), 1.25; CaCl\(_2\), 2; MgCl\(_2\), 2; NaHCO\(_3\), 26; dextrose, 10 (pH, 7.3–7.4; 290 mOsm). After trimming the chilled brain, a block containing the ventrobasal thalamus was glued onto a tissue-slicer stage with cyanoacrylate adhesive. Coronal slices of the thalamus were cut at 250–300 \(\mu\)m on a Leica VT1200S vibratome (Leica Biosystems, Nussloch, Germany) while the block was submerged in oxygenated, 1–4°C ACSF. Immediately after cutting, the slices were incubated at room temperature (22–24°C) in oxygenated ACSF.

#### Electrophysiological Recordings

For recording, slices were submerged in a Perspex chamber with a volume of 1.5 ml, fixed between two pieces of polypropylene mesh, and maintained at room temperature. The slices were continuously perfused by gravity with oxygenated ACSF at a flow rate of 2.5 ml/min controlled by a FR-50 flow valve (Harvard Apparatus, St. Laurent, QC, Canada). Individual neurons were visualized with the aid of differential interference contrast infrared videomicroscopy (Zeiss Axioskop FS, Carl Zeiss, Göttingen, Germany). The images were recorded with a Hamamatsu C2400 video camera system (Hamamatsu Photonics K.K., Hamamatsu, Japan). Patch pipettes were pulled from borosilicate glass (World Precision Instruments, Inc., Sarasota, FL) using a PP-83 two-stage electrode puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan) and filled with a solution containing (mM): K-glucuronate, 139; EGTA, 10; KCl, 6; NaCl, 4; MgCl\(_2\), 3; HEPES, 10; CaCl\(_2\), 0.5; adenosine-5’-triphosphate (disodium salt), 3; guanosine-5’-triphosphate (sodium salt), 0.3, titrated to pH 7.3–7.4 with 10% glucosone. Typical electrode resistances were 5–6 M\(\Omega\) and access resistance ranged from 10 to 20 M\(\Omega\). Whole cell patch-clamp recordings from ventrobasal thalamic neurons were performed in both current- and voltage-clamp modes with a HEKA EPC-7 amplifier (HEKA Elektronik Dr. Schulze GmbH, Lambrecht, Germany) via a Digidata 1322A 16 bit data acquisition system (Axon Instruments, Inc., Foster City, CA) using pCLAMP software (Axon Instruments, Inc.). The membrane currents were low-pass filtered (three-pole Bessel filter) at a frequency of 3 kHz and digitized at 10 kHz. Data were collected more than 10 min after whole cell access to allow the internal pipette solution to equilibrate with the neuron. Membrane potentials were corrected off-line for a liquid junction potential of \(-8\) mV.\(^37\) No leak subtraction was performed.

#### Data Analysis

Data were analyzed using ORIGIN 7 (OriginLab Corporation, Northampton, MA) and Prism 5 software (GraphPad, La Jolla, CA). To determine the IC\(_{50}\) and Hill coefficient, concentration-response curves were normalized and fitted using the Hill equation as follows:

\[
\frac{[L]}{[L]_{\text{max}}} = \frac{[C]^h}{([C]_{\text{IC}_{50}})^h + [C]^h}
\]  

\[\text{(1)}\]
where \( I \) represents the current measured in the presence of a given drug concentration; \( I_{\text{max}} \) is the control current measured in the absence of the drug; \([C]\) is the drug concentration; \( I_{\text{IC50}} \) is the half-maximal inhibitory concentration; and \( b \) is the Hill coefficient.

The conductance-voltage relationship for \( I_h \) steady-state activation was fitted by the Boltzmann equation:

\[
G_h / G_{h(\text{max})} = 1 / (1 + \exp((V - V_{0.5})/k))
\]  
(2)

where \( G_h \) is the \( I_h \) conductance (calculated as \( G_h = I / [V - V_t] \)); \( I \) amplitude of the \( I_h \) tail current following a hyperpolarizing step \([V]; V_t \text{ estimated } I_h \text{ reversal potential); } G_{h(\text{max})} \) is the maximum conductance obtained after the most hyperpolarizing step; \( V_{0.5} \) is the half-maximal activation potential; and \( k \) is the slope factor.

We estimated the reversal potential of \( I_h \) from the intersection of extrapolated linear regression fits of instantaneous voltage-current relationships at two different holding membrane potentials. The slopes of the plots were assumed to vary depending on the degree of activation of \( I_h \) and to intersect at the reversal potential of \( I_h \) where there is no driving force.

Data are presented as mean ± SEM unless mentioned otherwise; baseline membrane properties of all included neurons are given as mean ± SD as indicated. We used one-way ANOVA to test for concentration-dependent drug effects and comparisons of more than two groups. Comparisons between two groups were conducted with the use of a paired Student \( t \) test; a one-sample Student \( t \) test was used to test for differences of normalized data from baseline (i.e., a hypothetic mean of 1.0). Statistical tests were two-tailed and results were considered significant at \( \alpha = 0.05 \).

**Drugs and Chemicals**

Lidocaine HCl, tetrodotoxin, and CsCl were purchased from Sigma–Aldrich Canada Ltd. (Mississauga, ON, Canada). ZD7288 was obtained from Ascent Scientific (Princeton, NJ). BaCl\(_2\) was obtained from ICN Biomedicals (Aurora, OH). Lidocaine, tetrodotoxin, and ZD7288 were dissolved in fresh ACSF to prepare concentrated stock solutions stored at 4°C. Before application, required aliquots of the stock solutions were dissolved in ACSF to obtain the respective concentrations. All drugs were applied to the bath by switching from the control perfusate to ACSF containing a desired drug concentration. Recordings were conducted after 6 min of perfusion (approximately 2 ml/min) of the slices with a test solution except ZD7288 (20 min). All results reported reflect steady state responses.

**Results**

We investigated \( n = 62 \) thalamocortical neurons of the ventrobasal complex (ventral posterior lateral/medial nuclei). The neurons had an average (± SD) RMP of \(-67.2 ± 3.1 \) mV, consistent with the results of previous studies. When voltage-clamped at \(-68 \) mV, the neurons had an average (± SD) input resistance (\( R \)) of \( 271 ± 84 \) MΩ, determined from the responses to a 5 mV hyperpolarizing voltage step. Their average (± SD) membrane capacitance (\( C_l = \tau_m / R \)) was \( 197 ± 50 \) pF. All neurons voltage-dependently exhibited both the delay and oscillatory modes of operation characteristic for thalamocortical relay neurons (see Introduction, third paragraph). Accordingly, they responded with tonic repetitive firing to depolarizing current pulses from membrane potentials positive to approximately \(-60 \) mV, and, when depolarized from hyperpolarized membrane potentials less than approximately \(-70 \) mV, responded with burst firing, generated by a low-threshold spike (LTS; known to be mediated by \( I_f \); see Introduction, third paragraph) crowned by a burst of action potentials.

**Lidocaine Concentration-dependently Blocked \( I_h \) in Thalamocortical Neurons**

Hyperpolarization of neurons voltage-clamped at \(-68 \) mV induced an inwardly rectifying, noninactivating current consisting of an instantaneous component and a slow-activating component (fig. 1A), known to be generated by the inwardly rectifying K\(^+\) current, \( I_{Ki} \) (Kir), and the hyperpolarization-activated mixed cation current, \( I_h \), respectively. Extracellular application of 600 \( \mu \)M lidocaine inhibited \( I_h \) (calculated as the difference between the instantaneous current \( [I_{\text{inst}}] \) and the steady-state current \( [I_h] \) at the beginning and end of the voltage step, respectively) without affecting \( I_{Ki} \) \((n = 4; \) fig. 1, A and B). Lidocaine’s effects were mirrored by the specific \( I_h \) antagonist, ZD7288 (50 \( \mu \)M), which similarly blocked only the \( I_h \) component as predicted \((n = 4; \) fig. 1, C and D), whereas Cs\(^+\) (CsCl, 2 mM), a nonspecific \( I_h \) blocker, inhibited both \( I_h \) and \( I_{Ki} \) \((n = 4; \) fig. 1, E and F). Conversely, application of the \( I_{Ki} \) blocker, BaCl\(_2\) (0.1 mM), almost completely abolished this current and effectively unmasked \( I_h \) \((n = 5; \) fig. 2). Lidocaine reversibly blocked \( I_h \) in a concentration-dependent manner, with an \( IC_{50} \) of \( 72 ± 7 \) \( \mu \)M \((n = 4; \) ANOVA, \( P < 0.001 \)) and an estimated Hill coefficient of \( 1.19 ± 0.001 \) (fig. 3A; see Materials and Methods, Data Analysis sections). As illustrated in figure 4A, the activation curve rose between \(-58 \) and \(-128 \) mV, with a half-maximal activation potential \((V_{0.5}) \) of \(-94.6 ± 1.9 \) mV and a slope factor of \( 11.4 ± 1.0 \) \((n = 4)\), yielding an estimated maximum \( G_h \) in the range of

\( -271 ± 84 \) MΩ, determined from the responses to a 5 mV hyperpolarizing voltage step. Their average (± SD) membrane capacitance (\( C_l = \tau_m / R \)) was \( 197 ± 50 \) pF. All neurons voltage-dependently exhibited both the delay and oscillatory modes of operation characteristic for thalamocortical relay neurons (see Introduction, third paragraph). Accordingly, they responded with tonic repetitive firing to depolarizing current pulses from membrane potentials positive to approximately \(-60 \) mV, and, when depolarized from hyperpolarized membrane potentials less than approximately \(-70 \) mV, responded with burst firing, generated by a low-threshold spike (LTS; known to be mediated by \( I_f \); see Introduction, third paragraph) crowned by a burst of action potentials.

**Effects of Lidocaine on Biophysical Properties of \( I_h \)**

Because intrinsic and network thalamocortical oscillations are critically dependent on the activation and deactivation properties of \( I_h \), we further investigated whether lidocaine would alter these. To examine the conductance-voltage relationship for \( I_h \) steady-state activation, we measured \( I_h \) tail currents on repolarization to \(-78 \) mV (shown in fig. 3A; see Materials and Methods, Data Analysis sections). As illustrated in figure 4A, the activation curve rose between \(-58 \) and \(-128 \) mV, with a half-maximal activation potential \((V_{0.5}) \) of \(-94.6 ± 1.9 \) mV and a slope factor of \( 11.4 ± 1.0 \) \((n = 4)\), yielding an estimated maximum \( G_h \) in the range of
Fig. 1. Lidocaine blocked the hyperpolarization-activated cation current, $I_h$, in ventrobasal thalamocortical relay neurons without affecting the inwardly rectifying K$^+$ current, $I_{Kir}$ (A, C, E, and G). Representative current responses ($I$) of neurons voltage-clamped at $-68$ mV to 3-s hyperpolarizing voltage pulses injected in 10-mV increments (in A, ordinate labeled $E$) under control conditions and following application of either 600 $\mu$M lidocaine, 50 $\mu$M ZD7288, 2 mM CsCl, or 0.1 mM BaCl$_2$. Note the nonlinearly increasing amplitudes of current responses to successive hyperpolarizing voltage injections in neurons under control conditions, indicative of activation of an inwardly rectifying current. This current consisted of an instantaneous component, generated by $I_{Kir}$, and a slow-activating component, generated by $I_h$. The magnitude of $I_h$ was calculated as the difference between the instantaneous current at the beginning of each pulse ($I_{inst}$) and the steady-state current ($I_{ss}$) at the end of the pulse (A, arrows). (A) Lidocaine robustly blocked $I_h$ without affecting $I_{Kir}$. (C) The effects of lidocaine were mirrored by the specific $I_h$ antagonist, ZD7288. (E) In contrast, CsCl nonspecifically blocked both $I_h$ and $I_{Kir}$. (G) BaCl$_2$ almost completely blocked $I_{Kir}$, thereby unmasking $I_h$ at hyperpolarized potentials. Neurons exhibited recovery after washout (not shown) except after application of ZD7288. (B, D, F, and H) show the neurons’ $I_{inst}$ and $I_h$ currents plotted against the membrane potential at baseline (Control) and in the presence of either 600 $\mu$M lidocaine (B), 50 $\mu$M ZD7288 (D), 2 mM CsCl (F), or 0.1 mM BaCl$_2$ (H).
3–12 nS. Lidocaine (100 μM) did not significantly shift the $V_{0.5}$ ($-90.5 \pm 3.1$ mV; $n = 4; P = 0.11$), but decreased the slope factor to $7.6 \pm 1.1$ ($P = 0.02$; fig. 4A). These minor effects possibly reflected an improved quality of the voltage-clamp due to an increase in neuronal $R_i$.

We examined the rate of $I_h$ activation by stepping neurons to potentials from $-98$ to $-128$ mV (fig. 3A). The resulting kinetics were examined by fitting the activation phase of the current with a double-exponential function. The fast time constant decreased from 1,553 ± 510 ms at $-98$ mV to 274 ± 33 ms at $-128$ mV ($n = 4$; ANOVA, $P = 0.02$). Lidocaine (100 μM) had no significant effect on the fast time constant of $I_h$ activation in the voltage range from $-98$ to $-128$ mV ($n = 4$; each voltage tested, $P > 0.05$; fig. 4B).

Fig. 2. Reversal potential of $I_h$ in thalamocortical relay neurons. (A) Current responses ($I$) to 500-ms voltage pulses injected in 10-mV increments ($E$) of a neuron held at potentials ($V_h$) of $-68$ mV and $-88$ mV, respectively. Recordings were conducted in the presence of extracellular $\text{Ba}^{2+}$ (0.1 mM $\text{BaCl}_2$) to block the inwardly rectifying K$^+$ current, $I_{\text{KIR}}$. The voltage-current relationships of the instantaneous current at the beginning of each pulse ($I_{\text{inst}}$, fig. 1A; arrows) were determined at both potentials. (B) The $I_{\text{inst}}$ voltage-current data ($n = 5$ neurons) were fit with linear regression and the $I_h$ reversal potential ($V_r$) was estimated from the intersection of the two regression lines.

Fig. 3. Characteristics of lidocaine block of $I_h$ in thalamocortical relay neurons. (A) Representative current responses ($I$) of a neuron voltage-clamped at $-68$ mV to 3 s voltage pulses injected in 10-mV increments ($E$) under control conditions, after application of 100 μM lidocaine, and after 20 min washout (Recovery). Concentration ($B$) and voltage ($C$) dependences of lidocaine inhibition of $I_h$. Amplitudes of $I_h$ in $B$ (each concentration, $n = 4$; $n = 16$ neurons total; ANOVA, $P < 0.001$) and $C$ in the presence of lidocaine (100 μM in $C$; $n = 4$; ANOVA, $P > 0.05$) were normalized to those in control at the same test voltage ($-128$ mV in $C$). All recordings were performed in the presence of 0.1 mM $\text{BaCl}_2$. 

Lidocaine Blocks $I_h$ in Rat Thalamus

Because the \( I_h \) was still increasing slightly even at the end of a 3-s activation pulse, the slow time constant, especially at less negative potentials (fig. 3A), could not be estimated accurately; longer activation pulse durations compromised the whole cell patch.

We determined the rate of \( I_h \) deactivation by examining its tail current relaxation kinetics upon repolarization to −78 mV after a 3-s hyperpolarizing pulse to −128 mV. Repolarization to more depolarized potentials resulted in contamination of \( I_h \) tail currents with T type, low-threshold \( \text{Ca}^{2+} \) conductances (see Results section, first paragraph). Fitting the kinetics of \( I_h \) depolarization with a single-exponential function produced a deactivation time constant of 991 ± 17 ms (n = 4). Lidocaine (100 \( \mu \)M) substantially delayed \( I_h \) deactivation (fig. 4C), such that the deactivation time constant could not be estimated correctly in three of four neurons.

**Implications of Lidocaine’s Actions on \( I_h \) for Membrane Electrical Properties of Thalamocortical Neurons**

To investigate the implications of lidocaine’s actions on \( I_h \) for thalamocortical neurons’ membrane electrical properties, we performed a series of current-clamp experiments in neurons pretreated with the tonic \( \text{Na}^+ \) channel blocker, tetrodotoxin. At 600 nM, tetrodotoxin did not significantly alter passive membrane properties of neurons: the baseline \( R_i \), RMP, and \( C_i \) were 303 ± 21 M\( \Omega \), −65.9 ± 0.8 mV, and 206 ± 15 pF, respectively, compared with 277 ± 26 M\( \Omega \) (\( P = 0.22 \)), −66.4 ± 1.1 mV (\( P = 0.45 \)), and 201 ± 26 pF (\( P = 0.77 \); for all variables, n = 12) in the presence of tetrodotoxin. Application of tetrodotoxin also did not greatly change current-voltage relationships at potentials negative to −60 mV, but reduced the apparent \( R_i \) at depolarized potentials (not shown).

Application of lidocaine at concentrations blocking \( I_h \) produced a reversible increase in the \( R_i \) of neurons and a hyperpolarization of their RMP (table 1). These effects were concentration-dependent with a peak at 600 \( \mu \)M, but diminished in magnitude at 1 mM (fig. 5, A and B). Of note, application of 1 mM lidocaine initially (within the first 2 min) resulted in hyperpolarization of the RMP followed by its eventual depolarization to a steady-state value. The \( C_i \) was not significantly different from the baseline values over the range of 0.1–1 mM (data not shown), indicating a primary effect of lidocaine on membrane conductance (\( 1/R_i \)).

**Table 1. Effects of Lidocaine Compared with Those of the \( I_h \) Blockers, CsCl and ZD7288, on Membrane Electrical Properties of Ventrobasal Thalamocortical Relay Neurons**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( R_i ) (M( \Omega ))</th>
<th>RMP (mV)</th>
<th>( R_i ) (M( \Omega ))</th>
<th>P Value</th>
<th>RMP (mV)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>262 ± 50</td>
<td>−68.0 ± 1.7</td>
<td>556 ± 93 (212%)</td>
<td>0.02</td>
<td>−78.4 ± 1.9</td>
<td>0.005</td>
</tr>
<tr>
<td>Lidocaine + TTX*</td>
<td>207 ± 51</td>
<td>−71.0 ± 2.6</td>
<td>377 ± 91 (182%)</td>
<td>0.03</td>
<td>−78.8 ± 1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Lidocaine + BaCl(_2)*</td>
<td>260 ± 71</td>
<td>−62.3 ± 2.2</td>
<td>380 ± 110 (146%)</td>
<td>&lt;0.001</td>
<td>−67.8 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CsCl</td>
<td>183 ± 14</td>
<td>−66.3 ± 3.0</td>
<td>312 ± 43 (170%)</td>
<td>0.02</td>
<td>−78.7 ± 1.7</td>
<td>0.003</td>
</tr>
<tr>
<td>ZD7288</td>
<td>234 ± 47</td>
<td>−69.7 ± 1.1</td>
<td>417 ± 94 (178%)</td>
<td>0.03</td>
<td>−78.3 ± 1.9</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Summarized are the input resistance (\( R_i \)) and resting membrane potential (RMP) of ventrobasal thalamocortical relay neurons under baseline control conditions (with 600 nM tetrodotoxin [TTX] or 10.1 mM BaCl\(_2\) present in the superfusing extracellular solution) and following application of lidocaine (600 \( \mu \)M), CsCl (2 mM), or ZD7288 (50 \( \mu \)M). The average magnitude of increase in \( R_i \) is given in parentheses. Each row, n = 4 except for lidocaine + BaCl\(_2\) (n = 16).

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To define the effects of $I_h$ blockade by lidocaine on the active membrane properties of neurons, we conducted current-clamp experiments at potentials negative to $-45$ mV, corresponding to the activation range of $I_h$. Neurons current-clamped at $-62$ to $-64$ mV exhibited in their voltage responses to hyperpolarizing current pulses a typical inward (“anomalous”) rectification consisting of instantaneous and time-dependent components (fig. 5C). Bath application of lidocaine (600 μM) inhibited only the time-dependent, $I_h$-mediated inward rectification and produced an increase in the voltage responses to injected current pulses most pronounced at hyperpolarized potentials (n = 4). Current-voltage relationship analyses of the lidocaine-induced changes (fig. 5D) revealed inhibition of a conductance with an average reversal potential of $-58.1 \pm 0.9$ mV (n = 4) and implicated other conductance(s) in addition to $I_h$ and voltage-gated Na$^+$ currents. In addition to the previously mentioned effects, lidocaine also increased the latency of rebound LTSs (arrows, fig. 5C; calculated as the time required by the membrane potential to reach the LTS peak following the termination of the hyperpolarizing current pulse; see Results, first paragraph) from $128 \pm 5$ ms to $279 \pm 47$ ms (n = 4, $P = 0.04$). This increase occurred despite greater hyperpolarization responses, which would result in a larger population of deinactivated T-type Ca$^{2+}$ channels.

**Effects of Extracellular Cs$^+$ and ZD7288**

We compared the effects of lidocaine on the passive and active membrane properties of thalamocortical neurons with those of Cs$^+$ and ZD7288. Extracellular application of both CsCl (2 mM) and ZD7288 (50 μM) led to an increase in the $R_l$ of neurons, comparable with that produced by 600 μM lidocaine, as well as a significant hyperpolarizing shift in their RMP (table 1). In the current-clamp mode, extracellular Cs$^+$ reversibly inhibited both components of the inward rectification in the voltage responses of neurons current-clamped at $-62$ to $-66$ mV (n = 4; fig. 6) whereas ZD7288 irreversibly abolished only the time-dependent, $I_h$-mediated component (n = 4; fig. 7). Both Cs$^+$ and ZD7288 increased the voltage response magnitudes at potentials negative to approximately $-50$ mV. More depolarized holding potentials, from $-60$ to $-63$ mV and from $-55$ to $-56$ mV, were required to trigger rebound LTSs in three of four and in two of four neurons in the...
presence of Cs⁺ and ZD7288, respectively. However, even in those neurons whose holding potentials were depolarized, Cs⁺ and ZD7288 application both increased the LTS latencies (figs. 6 and 7). In addition to delaying the activation of rebound LTSs, ZD7288 also decreased the number of action potentials in the LTS-evoked bursts from 3.8 ± 0.6 to 2.0 ± 0.4 (n = 4, P = 0.006). In contrast, Cs⁺ had no effect on LTS burst firing.

Effects of Lidocaine on Firing Properties of Thalamocortical Neurons

We also examined the effects of lidocaine at concentrations blocking $I_h$ on firing properties of neurons not pretreated with tetrodotoxin. For this purpose, we used a current-clamp protocol to generate both tonic and rebound burst firing, applied in 2-min intervals to neurons constantly injected with a depolarizing current required to shift their membrane potential from RMP to −58 mV (associated with the tonic mode of firing that occurs in states of vigilance and wakefulness in vivo; see Introduction, third paragraph). As expected from its action on voltage-gated Na⁺ channels, lidocaine, at 100 μM, abolished tonic firing of Na⁺-dependent action potentials. However, the number of action potentials in the rebound LTS-evoked bursts decreased only slightly at this concentration, from 5.3 ± 0.3 to 4.3 ± 0.3 (n = 3; P < 0.001) (fig. 8A1). The burst discharges disappeared only at 600 μM, with the exception of the first spike (n = 4; fig. 8A2), which, consistent with our previous findings, was resistant to lidocaine in all neurons tested (but blocked by 600 nM tetrodotoxin; fig. 5C), raising the possibility that lidocaine’s potency for voltage-gated Na⁺ channel blockade in thalamocortical neurons might be lower than for its blockade of $I_h$. At 600 μM, lidocaine produced a significant (7–10 mV) depolarization of the holding membrane potential and triggered repetitive firing of high-threshold spikes in response to the depolarizing current pulses. Application of 1 mM lidocaine led neither to a depolarizing shift in the holding potential nor to firing of high-threshold spikes (n = 5; fig. 8A3), although the latter could be evoked by increasing the amplitude of the depolarizing pulse (not shown). Similar to the findings in neurons pretreated with tetrodotoxin (fig. 5C), lidocaine at all three concentrations (100 μM, 600 μM, and 1 mM) concentration-dependently and reversibly increased LTS latencies (fig. 8A1–3). Consistent with previous findings of others, we observed little effect of lidocaine on LTS magnitude.

Also similar to our results obtained in tetrodotoxin-pretreated neurons, application of lidocaine produced a revers-
ible increase in slope resistance in the range from approximately \(-50\) to \(-85\) mV, a hyperpolarization of the RMP (table 1), and suppression of the time-dependent inward rectification. The lidocaine-induced changes (600 \(\mu\)M) in the current-voltage relationships reflected inhibition of a conductance with a reversal potential of \(-66.5 \pm 2.4\) mV \((n = 3;\) fig. 8B shows the current-voltage curves of a representative neuron). Application of Ba\(^{2+}\) (0.1 mM; fig. 8C) shifted this value above \(-55\) mV \((n = 4)\), toward the reversal potential of \(I_h\). We found that the lidocaine-induced increase in \(R_i\) was smaller in the presence of Ba\(^{2+}\) than that observed at baseline or in the presence of tetrodotoxin (table 1). Collectively, these data implicate a K\(^{+}\) conductance (other than \(I_{Kir}\)) besides \(I_h\) in the actions of lidocaine at 600 \(\mu\)M.

**Discussion**

Here, we have demonstrated that lidocaine reversibly and voltage-independently inhibited the hyperpolarization-activated mixed cation current, \(I_h\), in rat ventrobasal thalamocortical relay neurons. Lidocaine blocked \(I_h\) with high efficacy (producing near-complete blockade; fig. 3B) and a potency (IC\(_{50}\), 72 \(\mu\)M) similar or higher in comparison with that associated with its best-known effect, voltage-gated Na\(^{+}\) channel blockade.\(^{10,49}\) Our findings in the thalamus are overall comparable with previous observations in the periphery, i.e., rat dorsal root ganglion neurons (IC\(_{50}\), 99 \(\mu\)M)\(^{11}\) and also cardiac (sinoatrial) myocytes (IC\(_{50}\), 38 \(\mu\)M).\(^{50}\)

The biophysical and pharmacologic properties of \(I_h\) in our experiments were similar to those previously reported in thalamocortical neurons\(^{42,51}\) and correspond well to those characteristic for the underlying HCN2 channel isoform dominant in these neurons.\(^{12}\) Lidocaine blocked the \(I_h\)-mediated time-dependent inward rectification without affecting the instantaneous inward rectification due to the K\(^{+}\) current, \(I_{Kir}\). In addition, lidocaine substantially delayed \(I_h\) deactivation while exhibiting no effects on the rate and voltage dependence of \(I_h\) activation. These observations suggest that lidocaine’s action is unlikely to reflect a primary effect on channel gating.

**Functional Consequences of \(I_h\) Inhibition for Thalamocortical Neurons**

Consistent with the identified role of \(I_h\) in determining membrane electrical properties,\(^ {12,28,29,52}\) its blockade by lidocaine was accompanied by a concentration-dependent hyperpolarization and led to large increases in the voltage responses to hyperpolarizing current pulses. The magnitude of lidocaine’s effects declined at 1 mM, suggesting that other
Fig. 8. Lidocaine altered firing properties of thalamocortical relay neurons. (A) Voltage responses \( E \) of neurons current-clamped at \(-58 \text{ mV}\) to injection of a 1-s depolarizing current pulse followed by a 1-s hyperpolarizing pulse \( I \) under control conditions (black traces) and in the presence of 100 \( \mu \text{M} \) (A1), 600 \( \mu \text{M} \) (A2), and 1 mM lidocaine (A3) (red traces). The right panels in A1–3 depict portions of the voltage responses at an expanded scale, together with the corresponding responses showing recovery after washout (green traces), containing rebound low-threshold \( \text{Ca}^{2+} \) spikes (LTSs; arrows) crowned by action potential bursts. Lidocaine at 100 \( \mu \text{M} \) abolished tonic firing of \( \text{Na}^{+} \)-dependent action potentials and increased LTS latencies while only slightly decreasing the number of action potentials in the rebound LTS-evoked bursts (A1). At 600 \( \mu \text{M} \), lidocaine depolarized the neuron from the holding potential of \(-58 \text{ mV}\) and triggered repetitively firing high-threshold spikes (HTSs; red traces/arrow) in response to the depolarizing current pulses, while further increasing LTS latencies and abolishing rebound burst discharges with the exception of the first spike in a burst (A2). 1 mM lidocaine had similar effects on the rebound bursts but produced neither a depolarizing shift in the holding potential nor firing of HTSs (A3). (B) Voltage responses \( E \) of a neuron at rest \((-65 \text{ mV}\)) measured at the end of the 1-s current injections and plotted against the magnitude of the injected current \( I \) at baseline (Control) and in the presence of 600 \( \mu \text{M} \) lidocaine. Lidocaine produced an increase in slope resistance in the range from approximately \(-50 \text{ mV}\) to \(-85 \text{ mV}\) and a hyperpolarization of the resting membrane potential. The lidocaine-induced current-voltage relationship changes reflected inhibition of a conductance with a reversal potential of approximately \(-63 \text{ mV}\). (C) Voltage responses of a neuron measured at the end of the 1-s current injections and plotted against the magnitude of the injected current at baseline (Control), in the presence of \( \text{BaCl}_2 \) (0.1 mM), and in the presence of 600 \( \mu \text{M} \) lidocaine plus \( \text{BaCl}_2 \) (0.1 mM). In the presence of \( \text{Ba}^{2+} \), lidocaine almost completely inhibited inward rectification; \( \text{Ba}^{2+} \) depolarized the reversal potential of the conductance blocked by lidocaine toward the reversal potential of \( I_h \) (see Results, last paragraph).
conductances counteracting the hyperpolarization and increase in $R_t$ were activated. This observation is in agreement with the lidocaine-induced depolarization at more than 3 mM previously reported in cultured dorsal root ganglion neurons. The precise mechanisms are unknown and have been speculated to involve blockade of ion channels and pumps playing a role in the maintenance of RMP.

In the current study, the effects of lidocaine on membrane potential critically depended on holding voltage. At potentials associated with the relay mode of operation (> approximately −60 mV; see Introduction), lidocaine, at 600 μM, depolarized neurons. Current-voltage analyses showed that the depolarization occurred due to the hyperpolarized reversal potential of the lidocaine-blocked conductance relative to the holding membrane potential. At the same time, 100 μM lidocaine did not depolarize neurons and produced a smaller increase in $R_t$ than expected based on the concentration dependence of the $I_h$ inhibition. Combined with the effects of Ba$^{2+}$ on reversal potential and $R_t$ changes, these observations implicate the contribution of a K$^+$ conductance (other than the inward rectifier, $I_{ks}$) blockade to lidocaine’s actions that is substantially increasing at 600 μM. In good agreement is the report that lidocaine inhibits the hTREK1 current underlying leak K$^+$ conductance, with an IC$_{50}$ of 180 μM. The lidocaine-induced depolarization increased neuronal excitability mediated by (under the conditions of Na$^+$-dependent action potential blockade) a high-threshold Ca$^{2+}$ conductance. In this regard, our findings support the hypothesis of Mulle et al., explaining the occurrence of dendritic high-threshold spikes in thalamocortical neurons in the presence of intracellular QX-314 (100 μM), a permanently charged lidocaine analog, as resulting from an increase in $R_t$ due to inhibition of persistent Na$^+$ and/or K$^+$ conductances. With regard to lidocaine’s concentration-dependent effects on passive membrane properties, it is of note that an older study with “blind” recordings in the ventral posterior lateral nucleus of Sprague-Dawley rats yielded some results at variance with the current findings on $R_t$, failing to find a statistically significant effect at 600 μM. Whereas the precise reason is unclear, differences in recording technique and associated quality, species, animal age, neuronal homogeneity, and/or a type II error (an increase in $R_t$ occurred in some neurons) may have contributed.

In the current investigations, we also found that blocking $I_h$, lidocaine concentration-dependently altered firing properties of thalamocortical neurons in the burst mode, which in vivo is associated with nonrapid eye movement sleep and drowsiness. Specifically, lidocaine increased the latency of rebound LTSs. Most likely, inhibition of $I_h$ tail currents, known to evoke hyperpolarization-activated membrane potential overshoots, accounts for these effects. Furthermore, at 100 μM, lidocaine reduced the number of Na$^+$ action potentials in LTS-evoked bursts. Our results are consistent with previous observations that both pharmacologic (ZD7288) and “electronic” (dynamic clamp) $I_h$ blockade increase LTS latency, partially suppress LTS-evoked bursts, and decrease the propensity of thalamocortical neurons to generate intrinsic δ oscillations. We would therefore predict that lidocaine blockade of $I_h$ despite hyperpolarization, will reduce the ability of thalamocortical neurons to produce intrinsic burst firing and δ oscillations. The fact that intracellular QX-314 (100 μM) inhibits intrinsic slow oscillatory activity in cat thalamocortical neurons in vivo supports this prediction.

Clinical Relevance of $I_h$ Inhibition by Lidocaine in Thalamocortical Neurons

In a discussion of the potential clinical relevance of the current findings it is important to note that results from in vitro animal investigations obviously cannot easily be translated to the in vivo domain without consideration of experimental limitations. For example, whereas our current work has a specific focus on intrinsic properties of single ventrobasal thalamocortical neurons, $I_h$ also is expressed in other neurons involved in thalamocortical networks, such as those in the thalamic reticular nucleus and cortex. Future studies using such approaches as multiunit and field potential recordings, imaging, and neural network modeling will help define the $I_h$-mediated effects of lidocaine on the entire thalamocortical system and aid in filling the gap between findings from single cells in brain slices and higher levels of organization (and ultimately, the human patient).

These considerations notwithstanding, numerous lines of evidence render it a plausible possibility that the mechanisms of systemic lidocaine’s concentration-dependent CNS effects involve varying degrees of thalamic $I_h$ inhibition, thereby affecting neuronal excitability and oscillatory behavior. For example, with regard to the higher (and presumably epileptogenic/CNS-toxic) concentrations producing close to maximal $I_h$ blockade in the current study, an absence of $I_h$ in thalamocortical neurons of HCN2-deficient (−/−) knockout mice produces abnormal synchronized (3–5 Hz) electroencephalographic oscillations and facilitates the occurrence of spike-and-wave discharges. In rat models, a decreased responsiveness of $I_h$ to cyclic adenosine monophosphate in the ventrobasal thalamus promotes epileptogenesis. Lidocaine-induced depolarization and Ca$^{2+}$-mediated increase in excitability at high concentrations may well play a part. Clearly, a body of future research is required to further elucidate these mechanisms.

In addition to its toxic effects on the CNS, systemic lidocaine...
caine, at low, subconvulsive plasma concentrations (ranging from approximately 1–7 μg/ml or approximately 4–30 μM), is efficacious in alleviating acute postoperative as well as chronic neuropathic pain in humans,4,5,62,63 and animal models.64 We recognize that extrapolation of ACSF concentrations from in vitro rodent studies to in vivo human plasma concentrations (where, among other factors, protein binding occurs and species differences play a role) requires caution. However, the therapeutic lidocaine concentration range in humans is near the lower end of that producing the I h inhibition in this study (e.g., approximately 23% suppression at 30 μM), which is noteworthy particularly because the current experiments were conducted at room temperature to facilitate stable recording conditions and slice viability.

Given the pivotal role of the ventrobasal thalamus in pain and analgesia, our observations raise the possibility that moderate inhibition of thalamic I h in the low micromolar range might represent a contributing mechanism for lidocaine’s systemic analgesic actions. Again, future studies are required to test this hypothesis.

**I h: An Emerging Anesthetic Drug Target in the Thalamus?**

In addition to shedding new light on the mechanisms of lidocaine’s supraspinal CNS effects, the current findings also emphasize on the role of I h as an emerging anesthetic drug target. For example, our findings with lidocaine, which has well-known general anesthetic properties (see Introduction),1,3 share some noteworthy similarities with those recently obtained with propofol.5 In thalamocortical neurons, propofol inhibited I h-HCN2 at clinically relevant concentrations (e.g., 36% at 5 μM; 23°C) and slowed I h activation. Consistent with our predictions on the in vitro implications of lidocaine’s I h blockade, propofol’s actions resulted in decreased regularity and frequency of δ oscillations in the neurons. Another example is ketamine, recently reported to inhibit I h in mouse cortical pyramidal neurons (approximately 29% at 20 μM; room temperature); in HCN1 knockout mice, ketamine showed a dramatically decreased hypnotic efficacy.65 In addition, there is growing evidence that the anesthetic mechanisms of volatile agents involve I h inhibition.66,67 A comprehensive review on the role of I h and HCN channels in anesthesia and other physiologic and pathologic conditions (including pain and epilepsy) has appeared recently.68

**Summary and Conclusions**

In this work, we have shown that lidocaine concentration-dependently inhibited I h in ventrobasal thalamocortical neurons at micromolar concentrations in vitro, with high efficacy and a potency similar or higher compared with that associated with its blockade of voltage-gated Na+ channels. By inhibiting I h, lidocaine profoundly altered membrane properties of neurons and reduced their ability to generate the rebound burst firing associated with slow oscillatory cerebral activity. Our findings provide new insight into the multiple overlapping mechanisms that underlie the complex array of concentration-dependent therapeutic and toxic effects that intravenous lidocaine exerts on the CNS and emphasize on the significance of I h as an emerging anesthetic drug target.

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**References**


44. Nayak TK, Harinath S, Nama S, Somasundaram K, Sikdar SK: Inhibition of human two-pore domain K + channel TREK1 by...
local anesthetic lidocaine: Negative cooperativity and half-of-sites saturation kinetics. Mol Pharmacol 2009; 76:903–17


