SUPPRESSION OF $I_h$ CONTRIBUTES TO PROPOFOL-INDUCED INHIBITION OF MOUSE CORTICAL PYRAMIDAL NEURONS

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ABSTRACT

The contributions of the hyperpolarization-activated current, \( I_h \), to generation of rhythmic activities are well-described for various central neurons, particularly in thalamocortical circuits. In the present study, we investigated effects of a general anesthetic, propofol, on native \( I_h \) in neurons of thalamus and cortex and on the corresponding cloned HCN channel subunits. Whole-cell voltage clamp recordings from mouse brain slices identified neuronal \( I_h \) currents with fast activation kinetics in neocortical pyramidal neurons and with slower kinetics in thalamocortical relay cells. Propofol inhibited the fast-activating \( I_h \) in cortical neurons at a clinically relevant concentration (5 µM); inhibition of \( I_h \) involved a hyperpolarizing shift in half-activation voltage (\( \Delta V_{1/2} \sim -9 \) mV) and a decrease in maximal available current (~36% inhibition, measured at -120 mV). With the slower form of \( I_h \) expressed in thalamocortical neurons, propofol had no effect on current activation or amplitude. In heterologous expression systems, 5 µM propofol caused a large shift in \( V_{1/2} \) and decrease in current amplitude in homomeric HCN1 and linked heteromeric HCN1-HCN2 channels, both of which activate with fast kinetics, but did not affect \( V_{1/2} \) or current amplitude of slowly-activating homomeric HCN2 channels. With GABA\( _A \) and glycine receptor-channels blocked, propofol caused membrane hyperpolarization and suppressed action potential discharge in cortical neurons; these effects were occluded by the \( I_h \) blocker, ZD-7288. In summary, these data indicate that propofol selectively inhibits HCN channels containing HCN1 subunits, such as those that mediate \( I_h \) in cortical pyramidal neurons – and they suggest that anesthetic actions of propofol may involve inhibition of cortical neurons and perhaps other HCN1-expressing cells.
INTRODUCTION

Propofol (2,6 di-isopropylphenol) is an intravenous anesthetic with a chemical structure distinct from any other anesthetic that has been gaining use for induction and maintenance of anesthesia in clinical practice over the last 10 years. Despite this growth in popularity as an anesthetic compound, its mechanisms of action remain incompletely understood. Effects of intravenous anesthetics, including propofol, are generally thought to be mediated by GABAergic mechanisms (Rudolph and Antkowiak, 2004; Trapani et al., 2000). Indeed, propofol-induced suppression of the withdrawal response to painful stimuli (i.e., its immobilizing action) was completely abolished in mice with a point mutation (N265M) engineered into the β3 subunit of the GABA_A receptor in order to remove its sensitivity to propofol (Jurd et al., 2003). Interestingly, although recovery from propofol-induced loss-of-righting reflex was accelerated in the β3(N265M) animals (Jurd et al., 2003), the loss-of-righting reflex itself was not blocked. This indicates that the direct hypnotic actions of the drug were relatively preserved in these GABA_A receptor knock-in mice (Jurd et al., 2003). The results with this mouse model are consistent with earlier pharmacological studies, which demonstrated that only the immobilizing, but not the hypnotic, effects of propofol are sensitive to systemic administration of GABA_A receptor blockers (Little et al., 2000; Sonner et al., 2003b).

Additional evidence indicates that propofol can mediate other clinically relevant actions independent of the GABA_A receptor, perhaps via alternative ionic mechanisms. For example, anticonvulsant actions of propofol, assayed as the ability of the drug to reduce epileptiform activity in rat hippocampal slices, are insensitive to the GABA_A receptor antagonist bicuculline (Ohmori et al., 2004). Anesthetic and anticonvulsant properties of propofol have been attributed to suppressive effects on a hyperpolarization-activated cationic current (I_h) in hippocampal pyramidal neurons (Funahashi et al., 2001; Higuchi et al., 2003). In addition, a recent study indicates that propofol can enhance
inhibitory input onto thalamocortical relay neurons, gating sensory activity through the thalamus, by blocking small conductance calcium-activated potassium (SK) channels in reticular thalamic neurons (Ying and Goldstein, 2005). Thus, the overall actions of propofol may involve modulation of ion channels in addition to GABA<sub>A</sub> receptors.

There is little detailed information as to which brain regions are critical for different endpoints of anesthesia, but direct or indirect depression of neurons in thalamocortical loops may provide a point of convergence for anesthetic actions, contributing to a sleep-like hypnotic state (Alkire et al., 2000). The pacemaker current, $I_h$, figures prominently in the firing behavior of neurons in thalamocortical circuits (Pape, 1996; Pape and McCormick, 1989), where it promotes spontaneous rhythmic activity like that associated with sleep states (Pape, 1996). We have demonstrated that native $I_h$ is strongly modulated by volatile anesthetics in motoneurons (Sirois et al., 1998; Sirois et al., 2002), cells which are implicated in anesthetic-induced immobilization (Sonner et al., 2003a). In addition, propofol inhibits $I_h$ in hippocampal pyramidal neurons and in the area postrema (Funahashi et al., 2004; Higuchi et al., 2003). This suggests that the channels underlying $I_h$ may represent a point of convergence for multiple classes of anesthetics in various neuronal groups associated with anesthetic actions.

It is now clear that HCN channels represent the molecular basis for native $I_h$ (Ludwig et al., 1998; Santoro et al., 1997). The four different HCN subunits exhibit distinct biophysical properties and all are expressed to some degree in the mammalian central nervous system (Monteggia et al., 2000; Moosmang et al., 1999; Santoro et al., 2000). Among these subunits, HCN1 and HCN2 are most prominently expressed in the CNS, with the HCN1 subunit more selectively localized than the slower HCN2. In the studies reported here, we examined effects of propofol on native $I_h$ in mouse thalamocortical and cortical neurons and on cloned HCN subunits (mHCN1 and mHCN2) expressed heterologously. We find that propofol selectively inhibits fast HCN1 subunit-containing channels, like those that mediate $I_h$ in cortical pyramidal neurons, but
does not diminish slower HCN2 channel currents, such as those in thalamocortical neurons.
MATERIALS AND METHODS

Patch clamp recordings from thalamic relay and cortical pyramidal neurons

Transverse brain slices from mice and rats of either sex (14-22 days old) were prepared, as described previously (Sirois et al., 1998; Talley et al., 2000). Animals were decapitated under ketamine/xylazine anesthesia. The brain was rapidly removed from the cranium and submerged in an ice-cold substituted Ringer’s solution bubbled with 95% O₂ and 5% CO₂. The substituted Ringer’s solution contained (in mM): 260 sucrose, 3 KCl, 5 MgCl₂, 1 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose, and 1 kynurenic acid (Aghajanian and Rasmussen, 1989). Slices (200 to 300 µm) were cut using a microslicer (DSK 1500E; Dosaka, Tokyo, Japan). Before recording, slices were incubated at 37°C for 1 h and then subsequently at room temperature in a normal Ringer’s solution (mM): 130 NaCl, 3 KCl, 2 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 glucose, bubbled with 95% O₂ and 5% CO₂.

Patch clamp electrodes were pulled from borosilicate glass (Warner Instruments) to a DC resistance of 3-5 MΩ and coated with Sylgard 184 (Dow Corning Corporation). Whole-cell recordings were obtained from visually identified pyramidal neurons in layer V of somatosensory cortex and from relay neurons in the ventrobasal thalamic nucleus by using infrared differential interference contrast (IR-DIC) microscopy (Zeiss Axioskop FS Plus, Germany) and an Axoclamp 200B amplifier (Axon Instruments). Cell capacitance and series resistance (Rs, typically <20 MΩ) were compensated using the amplifier circuits (typically ~70% Rs compensation). In addition, Rs was continuously monitored on an oscilloscope between test pulses by using the seal test function on the amplifier. Only cells with stable Rs were included and any small changes in Rs were corrected online by using the compensation circuits of the amplifier.
For voltage-clamp recordings, the pipette solution contained (in mM): 
120 KCH$_3$SO$_3$, 4 NaCl, 1 MgCl$_2$, 0.5 CaCl$_2$, 10 HEPES, 10 EGTA, 3 MgATP, 0.3 GTP-Tris, pH 7.2 and 280 mOsm. Current-clamp recordings were made with pipette solution containing (in mM): 17.5 KCl, 122.5 potassium gluconate, 1 MgCl$_2$, 9 NaCl, 10 HEPES, 0.2 EGTA, 3 Mg-ATP, 0.3 Tris-GTP, pH 7.2 and 272 mOsm. Recordings were obtained at room temperature (22-24°C) while slices were continuously superfused (~3-4 ml/min) with a solution containing (mM): 140 NaCl, 3 KCl, 2 MgCl$_2$, 2 CaCl$_2$, 10 HEPES and 10 glucose, pH 7.3. The properties of neuronal $I_h$ (i.e., maximal amplitude, voltage dependence) measured in slices using a HEPES-based bath solution equilibrated with room air (i.e., 21% O$_2$) are virtually identical to those obtained with a hyperoxic HEPES bath solution (100% O$_2$) or with normal Ringers solution bubbled with 95% O$_2$ and 5% CO$_2$ (data not shown). All data were corrected for a measured liquid junction potential of 8 mV.

In order to block $I_h$ in some experiments, ZD-7288 (50 µM; Tocris Cookson) was included in the pipette solution or CsCl was added to the bath (3 mM). We also included BaCl$_2$ (200 µM) in most voltage clamp experiments to inhibit inwardly-rectifying K$^+$ currents in cortical and thalamic neurons. Where noted, tetrodotoxin (TTX at 0.5 µM, Alomone Labs) was added to the perfusate to block action potentials and a bicuculline/strychnine cocktail (both at 30 µM; from Sigma) was added in order to block GABA$_A$ and glycine receptor channels. Propofol (Sigma, St. Louis) was prepared as a 100 mg/ml stock solution in ethanol and diluted in bath solution to the indicated concentrations; propofol was applied to neurons in the slice for ~5 min (5.1 ± 0.2 min), a point at which we found inhibition of $I_h$ in cortical neurons was stable and maximal. In a subset of experiments on thalamocortical cells, propofol was perfused for 20 min before effects on $I_h$ were assessed.
Voltage clamp recording of heterologously expressed mHCN channels

We obtained mHCN1 and mHCN2 from Drs. B. Santoro and S.A. Siegelbaum (Columbia Univ., NY). The concatemeric HCN1-HCN2 construct was made by using overlap extension PCR to produce a PshAI-Nhel fragment that spliced the final leucine of HCN1 directly in frame with the initiating methionine of HCN2 (Chen et al., 2005). The pGHE vector was used for oocyte expression and the pcDNA3 vector for expression in mammalian cells.

To prepare RNA for injection, in vitro transcription was performed with Nhel-linearized DNA (HCN1), Sphl-linearized DNA (HCN2) or Xbal-linearized DNA (HCN1-HCN2) using T7 RNA polymerase (Message Machine; Ambion, Houston, TX). Xenopus laevis oocytes (obtained from Drs. G. Kamatchi and H. Fang, Univ. Virginia) were injected with 46 nl of RNA (50–200 ng/µl) using a Nanoject microinjector (Drummond Scientific Co., PA). Following injection, oocytes were incubated at 17°C for 1–3 days in ND-96 solution, containing (mM): 96 NaCl, 2 KCl, 1.8 CaCl2, 1 MgCl2, and 5 HEPES, pH 7.5 that was supplemented with 5% (w/v) gentamycin sulfate. Whole cell currents were recorded from oocytes in solution, containing (mM): 107 KCl, 5 NaCl, 10 Hepes, 1 MgCl2, and 1 EGTA, pH 7.3, at room temperature with the two-microelectrode voltage clamp technique using a Warner OC-725B amplifier (Warner Instruments, Hamden, CT). Voltage recording and current injecting electrodes were filled with 3 M KCl (1–3 MΩ).

HEK 293 cells were cultured using standard procedures and transiently transfected with HCN channel constructs, together with a GFP plasmid (pGreenLantern; GIBCO) using Lipofectamine 2000 reagent (Invitrogen). Following transfection (1–2 days), whole cell recordings of HCN channel currents were obtained from HEK293 cells as described above for neuronal recordings, except that the bath KCl concentration was raised to 25 mM (substituted equimolar for NaCl) (also see Chen et al., 2005).
Data acquisition and analysis

Data were acquired using pCLAMP software (Axon Instruments) and a Digidata 1322A or a Digidata 1200 digitizer (Axon Instruments). For voltage-clamp recording, time-dependent hyperpolarization-activated currents ($I_h$, HCN) were evoked with incrementing ($\Delta -10$ mV) hyperpolarizing pulses (3-4 s) from a holding potential of -40 or -50 mV, followed immediately by a step to fixed potential (-90 or -100 mV) in order to obtain tail currents. Current amplitude at each potential was taken as the difference between ‘instantaneous’ currents, measured immediately after the capacitive transient, and the current at the end of hyperpolarizing voltage steps; maximal available current was determined at -120 mV or -130 mV. Tail currents were normalized, plotted as a function of the preceding hyperpolarization step voltage and fitted with Boltzmann curves for derivation of half-activation voltage ($V_{1/2}$) by using a least squares analysis and the ‘solver’ add-in of Excel (Microsoft). Time constants ($\tau$) were determined by fitting currents evoked during hyperpolarizing steps to a biexponential function. Results are presented as mean ± SEM. Data were analyzed statistically using one-way analysis of variance (ANOVA) or Students t-test, as indicated; post-hoc pairwise comparisons used Bonferroni’s correction of the t-test (Excel or SigmaStat). Differences in mean values were considered significant if $P<0.05$.

Histology

In all slice experiments, 0.2% biocytin (Sigma-Aldrich) was included in the patch electrode solution for subsequent morphological identification of recorded cells. Slices were fixed in 4% buffered paraformaldehyde solution for at least 24 h, and then rinsed and incubated for 30 min with 1% $H_2O_2$ and for 30 min with 0.5% Triton X-100. A two hour incubation with avidin-biotin complex was followed by incubation in a diaminobenzidine substrate kit (both from Vector Labs, Burlingame, CA). Finally, slices were mounted onto gelatin-subbed slides and left to air dry overnight. Slices were
dehydrated through graded ethanol and xylene and embedded in DPX mounting medium (BDH Laboratory Supplies, England); biocytin-stained neurons were visualized and photographed using an Axioskop microscope (Zeiss), equipped with a digital camera (Retiga 1300C, QImaging) and IPLab software (Scanalytics).
RESULTS

*Propofol differentially modulates native $I_h$ in neocortical pyramidal neurons and thalamocortical neurons*

We used whole cell patch clamp recording in mouse brain slices to characterize hyperpolarization-activated currents ($I_h$) and their modulation by propofol in thalamocortical and neocortical neurons. Representative examples of cell morphology and $I_h$ recorded from those neurons are depicted in Fig. 1. In a multipolar thalamocortical neuron from the ventral posteromedial nucleus (Fig. 1A), currents evoked by hyperpolarizing voltage steps activated in a voltage-and time-dependent manner as expected of $I_h$. In pyramidal neurons from somatosensory cortex (Fig. 1B), $I_h$ was also observed during hyperpolarizing voltage steps; in cortical neurons, however, $I_h$ activated with faster kinetics than in thalamocortical neurons. Biexponential fits to $I_h$ obtained at fixed membrane potential of -120 mV revealed a variable slow component of $I_h$ that was accompanied by a fast component that accounted for the majority of current in both cortical and thalamocortical neurons (>60%). In most cortical neurons, $I_h$ activated with a fast time constant ($\tau_f$) that was less than 200 ms ($\tau_f = 79.5 \pm 14.2$ ms; n=9 of 12 cortical neurons recorded). Activation kinetics were much slower ($\tau_f >$200 ms at -120 mV) in a subpopulation of cortical neurons ($\tau_f = 434.6 \pm 16.2$ ms; n=3) and in all thalamic neurons ($\tau_f = 338.9 \pm 32.1$ ms; n=10).

These distinct kinetic properties of $I_h$ typical of cortical and thalamic neurons are consistent with earlier recordings of $I_h$ from these two brain regions, and with known patterns of HCN subunit expression in mouse cortex and thalamus (e.g., see Santoro et al., 2000). In neocortical neurons, there is relatively high expression of fast-activating HCN1 subunits and moderate levels of slow-activating HCN2 subunits (Franz et al., 2000; Santoro et al., 2000). In thalamocortical neurons, HCN1 is barely detectable and the slower HCN2 and HCN4 isoforms predominate (Notomi and Shigemoto, 2004;
Santoro et al., 2000), with the majority of the current (80-90%) attributed to HCN2 (Ludwig et al., 2003).

We found that the intravenous general anesthetic, propofol, acts differently on $I_h$ in cortical and thalamic neurons. As exemplified in records from the representative cells of Fig. 1, propofol (5 μM, for ~5 min) had little effect on $I_h$ in thalamic neurons, but it caused a robust inhibition of $I_h$ in the group of cortical neurons with fast activation kinetics ($\tau_f < 200$ ms). The effect of propofol on $I_h$ may be seen more clearly as propofol-sensitive currents (Fig. 1, *right panels*), which were obtained by subtracting currents in the presence of propofol from those in control conditions. There was essentially no propofol-sensitive current in thalamic neurons but we observed a substantial propofol-sensitive current with time- and voltage dependence characteristic of $I_h$ in cortical neurons.

The inhibition by propofol of fast-activating $I_h$ in cortical neurons was manifest in two prominent effects: a shift in voltage-dependence of activation and a decrease in maximal available current. As depicted in Fig. 2A (*left*), analysis of tail currents evoked at -100 mV following hyperpolarizing voltage steps to different membrane potentials revealed a propofol-induced hyperpolarizing shift in the half-activation voltage ($V_{1/2}$) by -9 ± 0.9 mV (from -94.4 ± 1.0 mV to 103.6 ± 1.2 mV, n=9, p<0.001). In addition, as shown in $I$-$V$ relationships for $I_h$ in these cortical neurons (Fig. 2A, *right*), propofol reduced maximal time-dependent current at the end of hyperpolarizing steps to -120 mV by 35.9 ± 5.6 % (p<0.001); propofol also decreased input conductance at the holding potential by ~15%, as evident by the decreased slope of averaged ‘instantaneous’ I-$V$ curves (from 6.2 ± 0.2 nS to 5.3 ± 0.2 nS; p<0.005, see Fig. 2A, *inset*). We also noted that current activation kinetics were slowed by propofol ($\tau_f$ at -120 mV from 79.5 ± 14.2 ms to 112.5 ± 26.2 ms, n=9, p<0.05).

By contrast, propofol had no effect on $I_h$ voltage dependence (-98.3 ± 0.6 mV and -97.8 ± 0.9 mV in control and propofol; Fig. 2B, *left*), amplitude (0.5 ± 0.0% activation;
Fig. 2B, right) or on input conductance (2.6 ± 0.6 nS and 2.5 ± 0.5 nS in control and propofol) in thalamic neurons (n=5) or in the group of three cortical neurons with a slow form of $I_h$ (V½: -101.2 ± 2.2 mV and -100.1 ± 2.8 mV in control and propofol; amplitude inhibition: 3.7 ± 0.1%). To rule out any concerns with the slow equilibration of propofol in slices (Bieda and MacIver, 2004; Gredell et al., 2004), we confirmed that propofol remained without effect on V½ or maximal amplitude of $I_h$ after a 20 min exposure to propofol (V½: -103.9 ± 1.2 mV and -106.4 ± 1.9 mV in control and propofol; amplitude inhibition: 7.6 ± 3.3%; n=5, NS). It is noteworthy, however, that propofol did slow activation kinetics in all thalamocortical cells (τf at -120 mV from 338.9 ± 32.2 ms to 474.1 ± 81.2 ms, n=10, p<0.05), and this effect was fully evident even after only 5 min of propofol application (τf at -120 mV from 409.8 ± 39.2 ms to 637.5 ± 126.1 ms, n=5, p<0.05). These data indicate that although an action of propofol on activation kinetics can be discerned in thalamocortical neurons, the anesthetic does not modulate voltage dependence or maximal amplitude of $I_h$ under conditions identical to those in which the fast activating $I_h$ in cortical neuron is clearly inhibited.

We extended this analysis to motoneurons of the rat hypoglossal nucleus, which like mouse cortical pyramidal neurons, co-express high levels of HCN1 and moderate levels of HCN2 (Chen et al., 2005; Monteggia et al., 2000) and present a relatively fast form of $I_h$ (τf at -120 mV: 126.3 ± 9.5 ms, n=7). In these motoneurons, 5 µM propofol caused a hyperpolarizing shift in activation voltage ($\Delta V_{1/2} = -5.8 \pm 2.3$ mV, from -92.8 ± 2.4 mV to -98.6 ± 3.7 mV, p<0.05) and a decrease in maximal current amplitude (20.6 ± 6.0% inhibition, p<0.05). Thus, propofol modulated the fast-activating $I_h$ observed in rat motoneurons, although not quite as strongly as in mouse cortical neurons (~10 mV shift in V½ and ~40% inhibition of peak current).
Propofol differentially modulates HCN1 and HCN2 channel subunits at clinically relevant concentrations

The data presented above indicate that propofol inhibits current amplitude, slows activation kinetics and shifts the voltage dependence of fast-activating $I_h$ currents in mouse cortical neurons, but only modulates activation kinetics of the slower $I_h$ currents recorded from mouse thalamocortical cells. Since fast-activating HCN1 subunits are prominently expressed in cortex and slower activating HCN2 subunits are predominant in thalamus (Ludwig et al., 2003; Santoro et al., 2000), we considered the possibility that these effects represent differential modulation of channels containing HCN1 and HCN2 subunits.

To test this hypothesis, we recorded currents from cloned mHCN1 and mHCN2 channel subunits expressed in Xenopus oocytes (Fig. 3). As reported, these two mHCN subunits produce homomeric channels with different activation properties: mHCN2 currents activate more slowly and at more hyperpolarized potentials than mHCN1 currents (Biel et al., 1999; Kaupp and Seifert, 2001; Santoro and Tibbs, 1999). We found that channels containing these subunits also differed in their modulation by propofol. In oocytes expressing mHCN1 subunits, propofol (5 µM) caused a hyperpolarizing shift in voltage dependence of activation ($\Delta V_{1/2}$ of -14.8 ± 1.6 mV, from -86.3 ± 2.2 mV to -100.6 ± 1.3 mV, n=6, p<0.005) and a decrease in maximal current amplitude of 27.9 ± 4.0% (p<0.05; Fig. 3C). These effects are similar to those observed on native $I_h$ in cortical neurons. On the other hand, propofol induced little effect on voltage dependence or amplitude of mHCN2 channel currents expressed in oocytes ($V_{1/2}$: -94.4 ± 3.6 mV and -96.8 ± 3.5 mV in control and propofol; amplitude inhibition: 2.5 ± 2.0%; Fig. 3C). For both mHCN1 and mHCN2, propofol caused a slowing of current activation ($\tau_f$ at -120 mV from 66.0 ± 4.4 ms to 238.2 ± 16.1 ms, n=5; and from 304.6 ± 35.2 ms to 438.0 ± 71.0 ms, n=5; both p<0.05).
These data indicate that the intravenous anesthetic propofol differentially inhibits HCN1 and HCN2 homomeric channels expressed in Xenopus oocytes. We confirmed these results in a mammalian expression system. In HEK293 cells expressing HCN1 channels, propofol shifted $V_{1/2}$, decreased current amplitude and slowed activation kinetics ($\Delta V_{1/2}: -20.5 \pm 1.7$ mV; amplitude inhibition: $16.5 \pm 4.1\%$; $\tau_f$ at $-120$ mV from $22.4 \pm 5.5$ ms to $106.8 \pm 9.4$ ms; $n=7$, all $p<0.05$); these effects of propofol on HCN1 channels were significantly greater than on HCN2-expressing cells ($\Delta V_{1/2}: -5.0 \pm 0.4$ mV; amplitude inhibition: $2.5 \pm 2.8\%$; $n=9$, $p<0.05$), except for the slowing of current activation that was again prominent with HCN2 channels in HEK293 cells ($\tau_f$ at $-120$ mV from $225.8 \pm 29.1$ ms to $335.1 \pm 50.8$ ms; $n=8$, $p<0.01$). We have reported that distinct inhibitory effects of an inhaled anesthetic, halothane, on HCN1 and HCN2 homomeric channels reflect different basal properties of the channels that can be overcome by cAMP modulation (Chen et al., 2005). However, addition of saturating cAMP (50 $\mu$M) to pipettes used to record HCN2 currents in HEK293 cells did not enhance propofol effects on either $V_{1/2}$ or current amplitude (data not shown, $n=5$). Thus, cAMP did not uncover additional latent inhibition of HCN2 channels by propofol.

**Propofol inhibits heteromeric HCN1-HCN2 channels**

A number of previous studies have demonstrated that HCN subunits can form heteromeric channels (Chen et al., 2001a; Much et al., 2003; Ulens and Tytgat, 2001). Therefore, we prepared a linked mHCN1 and mHCN2 cDNA and expressed that construct in oocytes to test effects of propofol on heteromeric HCN channels (Fig. 3). By using this concatenated construct, we were able to record from a homogenous population of HCN1-HCN2 heterotetrameric channels with a defined 1:1 stoichiometry; this approach yields macroscopic currents nearly identical to those obtained by co-injecting HCN1 and HCN2 subunit mRNAs in equal proportions (Chen et al., 2001a; Ulens and Tytgat, 2001).
As expected, we found that the linked HCN1-HCN2 heteromeric channel currents displayed kinetic and voltage dependent properties intermediate to those of homomeric mHCN1 and mHCN2 channels (Fig. 3A, 3B) (Chen et al., 2001a; Ulens and Tytgat, 2001). Likewise, effects of propofol on linked mHCN1-HCN2 heteromeric channel currents were also intermediate between those of mHCN1 and mHCN2 channels, inducing a hyperpolarizing shift in $V_{1/2}$ of $-11.6 \pm 2.6$ mV (from $-88.2 \pm 1.3$ mV to $-100.2 \pm 2.2$ mV; $n=5$, $p<0.005$), a decrease of maximal current amplitude by $20.2 \pm 4.3\%$ ($p<0.05$; Fig. 3C); propofol also cause a slowing of HCN1-HCN2 current activation ($\tau_f$ at $-120$ mV from $105.7 \pm 6.1$ ms to $215.3 \pm 13.6$ ms, $n=5$, $p<0.005$). Again, we confirmed these results in HEK293 cells expressing HCN1-HCN2 channels ($\Delta V_{1/2}: -10.2 \pm 0.9$ mV; amplitude inhibition: $10.6 \pm 3.5\%; \tau_f$ at $-120$ mV from $71.5 \pm 12.8$ ms to $134.3 \pm 16.9$ ms; $n=8$, all $p<0.05$). Thus, effects of propofol on heteromeric HCN channels were also similar to those observed on $I_h$ from most cortical neurons. Moreover, these data indicate that propofol inhibits either homomeric or heteromeric channels that contain HCN1 subunits.

We tested effects of propofol on HCN1-HCN2 heteromeric channels expressed in oocytes over a range of concentrations that encompasses those achieved clinically (0.5-50 µM). Propofol induced a hyperpolarizing shift in the voltage dependence of activation (Fig. 3D, left) and suppressed amplitude of heteromeric HCN channel currents (Fig. 3D, right) in a dose-dependent manner. There was little effect of propofol at 0.5 µM but a significant shift in $V_{1/2}$ and a decrease in maximal current amplitude both at 5 µM ($\Delta V_{1/2}: -11.0 \pm 1.9$ mV; amplitude inhibition: $20.2 \pm 3.1\%, n=6$) and at 50 µM ($\Delta V_{1/2}: -16.4 \pm 1.4$ mV; amplitude inhibition: $29.3 \pm 4.3\%, n=5$). These results indicate that propofol inhibits heteromeric HCN channels in dose-dependent manner over a clinically relevant concentration range.
Propofol modulates thalamic and cortical neuron $I_h$ with fast activation kinetics indicative of channels containing HCN1 subunits

A summary of data relating activation kinetics of cloned HCN channels and native neuronal $I_h$ to effects of propofol on voltage dependence of activation and maximal current amplitude is provided in Fig. 4. As described above, activation kinetics of native $I_h$ currents in individual neurons and of HCN channel currents expressed in oocytes were analyzed by using biexponential fits to currents obtained at -120 mV. The time constant of the fast exponential component ($\tau_f$) from cortical and thalamic neurons was correlated with both the shift in $V_{1/2}$ and the decrease in maximal current amplitude by propofol, in a manner consistent with averaged data from mHCN channel currents expressed in Xenopus oocytes. Thus, propofol induced a hyperpolarizing shift in $V_{1/2}$ and inhibition of $I_h$ amplitude in all cells with fast activation kinetics ($\tau_f < 200$ ms, see vertical dashed line), including the majority of cortical neurons and oocytes expressing either mHCN1 or mHCN1-2. In all neurons with fast activating $I_h$, the shift in $V_{1/2}$ and the amplitude inhibition by propofol was greater than the largest effect of propofol in HCN2-expressing oocytes (indicated by horizontal dashed lines). On the other hand, there was little effect of propofol on $V_{1/2}$ or current amplitude of the slowly-activating $I_h$ in thalamic neurons, whose response resembled that observed in oocytes expressing mHCN2. Note, also, that the few cortical neurons that expressed a slow form of $I_h$ were also relatively insensitive to propofol (Fig. 4, see shaded triangles with $\tau_f > 200$ ms). These data indicate that native $I_h$ currents with fast kinetics, presumably those containing HCN1 subunits, are subject to these inhibitory actions of propofol.

Propofol differentially modulates instantaneous HCN channel current

An instantaneous component of current, in addition to the voltage- and time-dependent component, has been observed in recordings from cloned HCN channels (Ludwig et al., 1998; Macri and Accili, 2004; Mistrik et al., 2005; Proenza et al., 2002). This current component represents tonic activation of HCN channels at holding potentials
depolarized to the threshold for voltage-dependent activation, and at least for HCN2 channels, can represent ~10% of the total available current (Chen et al., 2001b; Decher et al., 2004). We therefore tested if propofol inhibits instantaneous currents from HCN channels, and if it does so in a subunit-specific manner. In HEK293 cells expressing HCN1, HCN2 and HCN1-HCN2 we measured input conductance as the slope of I-V relationships from instantaneous currents (i.e., measured immediately after the capacitive transient and before development of time-dependent currents) evoked by hyperpolarizing voltage steps from a holding potential of -40 mV. As is evident in Fig. 5, instantaneous currents generated from all three HCN constructs were inhibited by 3 mM CsCl, an HCN channel blocker. Importantly, and consistent with the differential effects we observed on voltage- and time-dependent HCN currents, propofol inhibited tonically active currents from HCN1 and HCN1-HCN2 channels, but had no effect on instantaneous HCN2 channel currents. When expressed relative to the Cs⁺-sensitive input conductance (a measure of the tonically-active HCN current), propofol inhibited ~50% of instantaneous HCN1 current, ~30% of instantaneous HCN1-HCN2 current but only ~5% of the instantaneous HCN2 current. These data confirm earlier observations of tonically active HCN channel currents at depolarized membrane potentials (Ludwig et al., 1998; Macri and Accili, 2004; Mistrik et al., 2005; Proenza et al., 2002), and they demonstrate that propofol robustly and preferentially inhibits tonic currents from channels containing HCN1 subunits.

**Propofol causes membrane hyperpolarization and decreases excitability in cortical neurons by inhibition of I_h**

We next asked, for neocortical neurons where mHCN1 transcripts are prominent and where we observed potent inhibition of I_h by propofol, whether propofol-induced modulation of I_h results in a significant inhibition of cortical neuron excitability. In order to determine effects of propofol on cell excitability and resting potential, we performed continuous recordings of voltage responses elicited by rectangular depolarizing current
injection, in the presence of GABA_A and glycine receptor antagonists (bicuculline and strychnine, both at 30 µM). As shown in Fig. 6 for a representative cortical pyramidal neuron, propofol caused hyperpolarization of resting membrane potential that averaged 4.1 ± 0.1 mV (n=9) and it decreased cell excitability, even when membrane potential was returned to control levels by DC current injection. On average, propofol increased the current necessary to induce action potential discharge from 0.44 ± 0.02 nA to 0.72 ± 0.02 nA in 5 µM propofol (n=5, p<0.05) and to 0.54 ± 0.02 nA in propofol when membrane potential was returned to control by depolarizing DC current injection.

To confirm that the propofol-induced hyperpolarization of membrane potential was caused by suppression of I_h, we studied cortical neurons with or without ZD-7288 (50 µM), a potent I_h channel antagonist (Bosmith et al., 1993), in the pipette solution (Fig. 7). Again, recordings were performed in the presence of bicuculline and strychnine to block GABA_A and glycine channels. In a cell recorded without ZD-7288 in the pipette (Fig. 7A, left), propofol induced a membrane hyperpolarization, as described above (~4 mV; see open bar in Fig. 7D for averaged data). Propofol also increased input resistance (~9%, from 142.5 ± 5.5 MΩ to 154.5 ± 5.9 MΩ; n=8, p<0.005) and eliminated the rebound action potential discharge following the hyperpolarizing current pulse (Fig. 7A, right). Note that the depolarizing ‘voltage sag’ (arrow) and rebound potential, which correspond to activation and deactivation of I_h, respectively were not eliminated by propofol. This likely reflects the partial nature of propofol-induced inhibition of I_h, as well as enhanced activation of the residual I_h during the current pulse as a result of the membrane hyperpolarization and increased R_N that also accompanied propofol actions.

In cells recorded with 50 µM ZD-7288 in the pipette solution, we observed a time-dependent increase in input resistance (~19%, from 115.1 ± 1.0 MΩ to 136.7 ± 2.7 MΩ; n=4, p<0.05) and hyperpolarization of membrane potential (Fig. 6B, left) that proceeded for ~5 minutes following whole cell access; after reaching a steady-state, membrane potential in cells recorded with ZD-7288 was hyperpolarized ~12 mV relative
to control cells (-69.9 ± 1.6 mV vs. -58.3 ± 3.1 mV; n=4 and n=8, p<0.05; see Fig. 7C). The time course of membrane hyperpolarization mirrors the time-dependent, and essentially complete, inhibition of $I_h$ by intracellular ZD-7288 measured under voltage clamp in these neurons (data not shown). In the current clamp recordings of Fig. 7B, the abolition of $I_h$ by ZD-7288 is evident by the loss of the depolarizing ‘voltage sag’ (arrow) and rebound potential (compare voltage responses at 0 and 6 min, Fig. 7B, right).

Consistent with the idea that propofol causes membrane hyperpolarization by inhibition of $I_h$, we found that propofol had no effect on membrane potential or input resistance when $I_h$ was eliminated by intracellular ZD-7288, (Fig. 7B). As is clear from averaged results presented in Figs. 7C & 7D, the $I_h$ blocker ZD-7288 itself caused a significant hyperpolarization (Fig. 7C) and it occluded further effects on membrane potential by propofol (Fig. 7D).

We found that the $I_h$ blocker Cs$^+$ (3 mM, bath applied) also caused membrane hyperpolarization (-7.6 ± 1.6 mV) and strongly increased $R_N$ (~110%, from 121.5 ± 11.6 MΩ to 250.4 ± 34.0 MΩ; p<0.05) in individual cortical neurons (n=4). Both Cs$^+$ and ZD-7288 caused nearly identical decreases in $I_h$ and cortical neuron input conductance (~50%) under voltage clamp when the compounds were applied in the bath in the presence of 200 µM Ba$^{2+}$ (data not shown) So, the larger increase in $R_N$ by Cs$^+$ in these current clamp experiments, by comparison to ZD-7288, likely reflects two factors: additional block by Cs$^+$ of inwardly-rectifying K$^+$ currents and partial block of $I_h$ by intracellular ZD-7288 at the earliest time point in the recording. Unfortunately, because cortical neurons became increasingly unstable in the continued presence of Cs$^+$, even in the presence of TTX and a glutamate receptor blocker (kynurenate, 1 mM), we were unable to test an effect of propofol after blocking $I_h$ with Cs$^+$.

Overall, these data indicate that $I_h$ contributes a substantial persistent inward current near resting membrane potential in cortical pyramidal neurons, and that inhibition
of $I_h$ by propofol can decrease excitability and alter electroresponsive properties of these neurons.
DISCUSSION

In this study, we demonstrated that propofol inhibits a form of $I_h$ with relatively fast activation kinetics ($\tau_f < 200$ ms) that is expressed in mouse cortical pyramidal neurons or rat motoneurons. The inhibition of neuronal $I_h$ by propofol was evident at a clinically relevant concentration (5 µM) and involved a hyperpolarizing shift in voltage dependence of activation, a decrease in maximal available current and a slowing of activation kinetics. On the other hand, propofol had no effect on $V_{1/2}$ of activation or maximal current amplitude of a slower activating form of $I_h$ expressed in thalamocortical relay neurons ($\tau_f > 200$ ms), although kinetic slowing was evident. The different kinetics of $I_h$ observed in mouse cortical and thalamocortical neurons agrees with prior electrophysiological work (Santoro et al., 2000); it is also consistent with greater expression of fast-activating HCN1 subunits in mouse cortex (particularly in layer V pyramidal neurons) and with higher densities of slower activating HCN2 and HCN4 subunits in thalamic relay nuclei (Santoro et al., 2000). Accordingly, propofol modulated cloned homomeric HCN1 or heteromeric HCN1-HCN2 channel currents in ways reminiscent of its effects on $I_h$ in cortical neurons, causing a negative shift in $V_{1/2}$, decreasing maximal current amplitude and slowing activation kinetics. Also consistent with actions of the anesthetic on native $I_h$ in thalamocortical neurons, we found that cloned HCN2 homomeric channels were relatively insensitive to propofol, showing only kinetic slowing with no change in voltage dependence or maximal amplitude. Propofol suppressed excitability in cortical neurons, causing membrane hyperpolarization and decreasing action potential discharge. These actions of propofol were due to inhibition of $I_h$ since they persisted when GABA$_A$ and glycine receptors were blocked, but were completely occluded when $I_h$ was inhibited by ZD-7288. In sum, these results indicate that modulation of $I_h$ by propofol in cortical pyramidal cells and motoneurons, and
perhaps in other HCN1-expressing cells, may contribute to its GABA-independent anesthetic and/or anticonvulsant actions (Higuchi et al., 2003).

Any suggestion of a role for $I_h$ inhibition in the clinical actions of propofol presupposes modulation of the channels over a concentration range that is achieved clinically. In this respect, circulating concentrations of propofol at which 50% of patients fail to respond to verbal command and skin incision are 3.3 µg/ml (18 µM) and 15.2 µg/ml (85 µM), respectively (Smith et al., 1994). Since >95% of propofol is bound to serum proteins, free fractions of propofol that produce these anesthetic actions are expected to range from 1 µM to 4.5 µM, but may be as high as 10 µM (Shyr et al., 1995). We showed that inhibitory effects of propofol on cortical $I_h$ and on HCN1 subunit-containing channels occur within this clinically relevant concentration range, with near maximal effects at 5 µM. If access of the drug to the slice is limited, as has been suggested (Bieda and MacIver, 2004; Gredell et al., 2004), the actual effective concentration of propofol at the channel may be even lower than the bath concentrations we report.

We found a strong inhibition of $I_h$ by propofol in pyramidal neurons of the neocortex similar to that previously reported in CA1 hippocampal pyramidal neurons, although higher concentrations of propofol (50 µM to 1 mM) were necessary for propofol actions on hippocampal $I_h$ (Funahashi et al., 2001; Funahashi et al., 2004; Higuchi et al., 2003). The reason for this different propofol sensitivity in pyramidal neurons from neocortex and hippocampus, despite generally similar HCN expression and $I_h$ properties in the two cell types, remains to be determined. As with layer V neocortical neurons, CA1 hippocampal pyramidal neurons appear to express HCN1 predominantly, along with HCN2, and a corresponding native $I_h$ with fast activation kinetics (fast activation $\tau < 100$ ms at $\sim -$100 mV) (Santoro et al., 2000). Given joint expression of both HCN1 and HCN2 subunits in cortical and hippocampal pyramidal neurons, it is likely that $I_h$ in these cells includes a contribution from homomeric HCN channels as well as heteromeric HCN1-
HCN2 channels. Importantly, we showed that these heteromeric HCN1-HCN2 channels also produce currents with fast kinetics that are modulated by propofol.

We found that propofol caused membrane hyperpolarization near resting membrane potential (i.e., at -60 mV) and decreased excitability in neocortical neurons in a ZD-7288-sensitive manner, as also observed in hippocampal CA1 pyramidal neurons (Higuchi et al., 2003). This ZD-7288-sensitive action of propofol at relatively depolarized membrane potentials likely involves inhibition of a tonic component of $I_h$ since we find that only ~5% of the voltage- and time-dependent current component is available at -60 mV (e.g. see Fig. 2A). Consistent with this, we showed that propofol can robustly inhibit tonic currents from HCN1 subunit-containing channels. In addition, it is possible that inhibition of dendritic $I_h$ by propofol could enhance temporal summation of synaptic inputs in those neurons, especially those impinging on distal dendrites where $I_h$ channels are most dense (Magee, 2000; Migliore and Shepherd, 2002). Other ionic mechanisms likely also contribute to effects of propofol on cortical neuron excitability. For example, propofol can inhibit apamin-sensitive calcium-activated K$^+$ currents (Ying and Goldstein, 2005) and it enhances synaptic as well as tonically active GABA$\_\alpha$-currents in neocortical and hippocampal neurons (Bieda and MacIver, 2004; Orser et al., 1994). So, it seems that multiple ion channel targets – intrinsic and synaptic, acting tonically and phasically – could contribute to complex integrated actions of propofol in cortical neurons and elsewhere.

As mentioned above (see Introduction), although some anesthetic actions of propofol certainly involve enhancement of GABA$\_\alpha$ receptor (Jurd et al., 2003), existing pharmacological and genetic evidence leave open the possibility that other targets may also be important. In this respect, a role for HCN channels in hypnotic or amnestic effects of general anesthetics is intriguing. Thalamocortical circuits are implicated in the sleep-like actions of anesthetics (Alkire et al., 2000) and inhibition of $I_h$ in thalamic relay neurons and cortical neurons is associated with sleep-like rhythmic network activity.
(Pape, 1996; Pape and Mccormick, 1989). Our data suggest that any induction by propofol of a sleep-like state that involves actions on $I_h$ within thalamocortical circuits will engage primarily the cortical neuron component, where its actions on $I_h$ were most prominent. However, it is also possible that the propofol-induced slowing of $I_h$ activation kinetics we observed in cortical and thalamocortical relay neurons could also modulate rhythmic thalamocortical activity. In addition, $I_h$ underlies a ‘pacemaker’ current for generating the theta-like oscillations in entorhinal pyramidal neurons that provide a synchronizing mechanism important in memory formation (Dickson et al., 2000). Inhibition of cortical neuronal $I_h$ by propofol could disrupt such network coherence and thereby contribute to amnestic actions of the drug.

In conclusion, we have found that propofol inhibits the neuronal pacemaker current, $I_h$, in cortical pyramidal neurons at clinically relevant concentrations. These currents contribute to setting membrane potential and input resistance, they modulate dendritic integration and they are crucial for multiple cortical rhythms (Pape, 1996). These data are consistent with accumulating evidence that general anesthetics do not simply provide widespread suppression of neuronal excitability. Rather, it is likely that anesthetic drugs target multiple distinct types of ion channel at specific sites where they commandeer certain aspects of normal neuronal activity patterns in order to generate the anesthetic state. The precise contribution of HCN channel inhibition to overall anesthetic actions in cortical neurons will require modeling in simulated networks or experiments in animals with targeted disruption of HCN subunits.
REFERENCES


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FIGURE LEGENDS

Figure 1. *Propofol inhibits I_h in cortical pyramidal cells but not in thalamocortical relay neurons.*

A. Photomicrograph of a biocytin-stained thalamocortical neuron recorded from the ventral posteromedial nucleus.  B. Voltage clamp recordings of I_h (from the cell depicted in A) under control conditions (*left*) and in 5 µM propofol (*middle*). Subtracted currents (control - propofol) reveal the propofol-sensitive current (*right*). The voltage clamp pulse protocol is depicted in the *Inset* and is typical of that used throughout these studies (conditioning voltage steps were always 3-4 s in duration). C. Photomicrograph of a biocytin-stained pyramidal neuron recorded from somatosensory cortex. D. Voltage clamp recordings of I_h (from the cell depicted in C) under the indicated conditions. Note the slower kinetics of I_h in the thalamic neuron and that propofol only inhibited the faster-activating current in the cortical neuron.

Figure 2. *Modulation of fast-activating I_h in cortical neurons includes a shift in activation gating and a decrease in maximal available current.*

The effect of propofol was determined on (A) fast-activating I_h in cortical pyramidal neurons (τ_f < 200 ms at -120 mV; n=9/12 neurons) and on (B) slower-activating I_h in thalamocortical neurons (τ_f > 200 ms at -120 mV; n=5/5 neurons). Under control conditions (*filled squares*) and in the presence of propofol (*open triangles*), normalized tail currents were obtained following incrementing hyperpolarizing steps, averaged and fitted with Boltzmann curves (*left*). Likewise, time-dependent currents were measured at the end of each voltage step in control and propofol and plotted as a function of membrane potential. Propofol induced ~9 mV shift in V½ of I_h activation and ~36% decrease in I_h amplitude (at -120 mV) in cortical neurons but had no effect on I_h in thalamocortical cells. The *inset* in Fig. 2B plots ‘instantaneous’ currents evoked by
hyperpolarizing voltage steps in cortical neurons, measured immediately after the capacitive transient but before development of the time-dependent current; propofol decreased the slope of the instantaneous I-V curve (i.e., the input conductance).

Figure 3. Propofol modulates HCN channels containing the mHCN1 subunit.
A. Sample currents from Xenopus oocytes expressing mHCN1, mHCN2 or a tandem-linked heteromeric mHCN1-HCN2 construct evoked by hyperpolarizing voltage steps from -40 mV to -130 mV before, during and following exposure to propofol (5 µM); conditioning voltage steps were of different duration for the three constructs (2, 3, & 4 s for HCN1, HCN1-HCN2 and HCN2, respectively) and followed by a step to -90 mV for tail current analysis. B. Activation curves were fitted according to the Boltzmann equation to normalized, averaged tail currents (left) and averaged steady-state I-V curves were obtained from currents at the end of the voltage steps (right) under control conditions (open squares), during exposure to 5 µM propofol (filled squares) and following recovery (open triangles) for HCN1, HCN2 or a linked HCN1-HCN2 construct. C. The effect of propofol (5 µM) on half-activation potential (ΔV½) and maximal current amplitude was determined in individual oocytes expressing the indicated constructs under control conditions and averaged (± SEM, n=7, 8 and 6 for HCN1, HCN2 and HCN1-HCN2). *, significantly different from HCN2, p<0.05 by ANOVA.

D. Concentration-response data for propofol (0.5, 5 and 50 µM) modulation of HCN1-HCN2 heteromeric channel currents in oocytes; a significant hyperpolarizing shift in V½ (left) and inhibition of current amplitude (right) were already evident at only 5 µM propofol. *, significantly different from 0.5 µM, p<0.05 by ANOVA.

Figure 4. Propofol modulates fast-activating HCN channels and neuronal Ih.
The relationship between activation kinetics of neuronal Ih or HCN currents and the shift in V½ (A) or inhibition of maximal current amplitude (B) induced by propofol (5 µM).
Activation data were obtained from biexponential fits at -120 mV and the time constant ($\tau_f$) describing the fastest (and largest) current component was plotted together with data from individual neurons or averaged data ($\pm$ SEM) from oocytes expressing the indicated constructs. In most cortical neurons (shaded diamonds), or in oocytes expressing HCN1 and HCN1-HCN2 channels, currents activated with a $\tau_f < 200$ ms (indicated by the dashed vertical lines). In these cells, propofol induced a shift in $V_{1/2}$ or a current inhibition that was greater than the largest effect measured in all but one thalamic neuron (open triangles) or in any HCN2-expressing oocyte (indicated by the dashed horizontal lines; $\Delta V_{1/2} = 4.5$ mV: inhibition = 12.4%). Note that there was little effect of propofol on the three cortical neurons with a slow form of $I_h$ ($\tau_f > 200$ ms).

**Figure 5. Propofol inhibits a Cs$^+$-sensitive instantaneous HCN channel current.**

**A.** Instantaneous I-V relationships were obtained from a holding potential of -40 mV in HEK293 cells expressing HCN1 (left), HCN1-HCN2 (middle) and HCN2 (right) under control conditions (circles), in the presence of 5 µM propofol (diamonds) and 3 mM CsCl (squares). Solid lines represent linear fits through averaged data ($\pm$ SEM; n=7, n=10 and n=9 for HCN1, HCN2 and HCN1-HCN2), representing the input conductance at -40 mV.

**B.** Input conductance was determined from instantaneous I-V curves in individual cells expressing the HCN channel constructs, and averaged for each condition as indicated. Propofol decreased input conductance in HCN1 and HCN1-HCN2 expressing cells, but not in cells expressing HCN2; cesium inhibited the tonic current in all HCN transfected cells (*, P<0.05 vs. control).

**Figure 6. Propofol causes membrane hyperpolarization and decreased excitability in cortical neurons independent of the GABA$\_A$ receptor.**

**A.** Whole cell current-clamp recording of cortical neuron membrane potential before and during bath application of propofol (5 µM), in the continued presence of bicuculline and
strychnine (both at 30 µM). At a point near the maximal hyperpolarization induced by propofol (Δ ~-4 mV), membrane potential was restored to control levels (~-60 mV) by using depolarizing DC current injection. Vertical deflections are truncated membrane responses to periodic injections of depolarizing current pulses. B. Spike firing responses to rectangular depolarizing current pulses (0.5 nA to 0.9 nA; from points indicated in A) are expanded. By comparison to control (a), depolarizing current pulses of greater magnitude were required to evoke spike firing in the presence of propofol, whether the cell was returned to control membrane potential (b) or from the maximal propofol-induced hyperpolarization (c).

**Figure 7. Inhibition of I_h accounts for membrane hyperpolarization by propofol in cortical neurons.**

Whole cell current-clamp recordings were performed to test effects of the I_h blocker, ZD-7288 on cortical neuron membrane potential and response to propofol (5 µM), in the continued presence of bicuculline and strychnine (both at 30 µM). A. Representative time course of membrane hyperpolarization (from -64 mV to -68 mV) induced by propofol in a cortical neuron recorded without ZD-7288 (left); voltage responses to hyperpolarizing current pulses include a ‘sag’ characteristic of I_h activation (arrow) and a rebound spike that is absent in the presence of propofol (right). B. Time course of membrane potential in a representative cortical neuron recorded with a pipette containing ZD-7288 (50 µM). A progressive hyperpolarization (from -66 mV to -74 mV) began immediately after obtaining whole cell access (left), after which application of propofol had no additional effect on membrane potential. Note that the depolarizing ‘sag’ (arrow) and rebound potential that was evident in initial voltage responses to hyperpolarizing current pulses (0 min) disappeared in conjunction with the membrane hyperpolarization (6 min), indicating that I_h was blocked by ZD-7288 at the time of propofol application (right). C. Averaged membrane potential in cortical neurons recorded with normal
internal solution and with pipettes containing ZD-7288. (*, p<0.05 by unpaired t-test)

D. Averaged propofol-induced hyperpolarization in cortical neurons recorded with normal internal solution and with pipettes containing ZD-7288. (*, p<0.001 by unpaired t-test)
Figure 1
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A. Thalamocortical neuron

B. Control, Propofol, Subtracted traces

C. Cortical pyramidal neuron

D. Control, Propofol, Subtracted traces
Figure 2
Chen et al.

**A** cortical pyramidal neurons

**B** thalamocortical neurons
Figure 3
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A

HCN1
control  propofol  wash

HCN2

HCN1-HCN2

B

propofol modulation of HCN channels

C

D

HCN1-HCN2 propofol dose-response
Figure 4
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Figure 5
Chen et al.

A

HCN1

membrane potential (mV)

-140 -120 -100 -80 -60 -40

instantaneous (pA)

-140 -120 -100 -80 -60 -40

HCN1-HCN2

membrane potential (mV)

-140 -120 -100 -80 -60 -40

instantaneous (pA)

-140 -120 -100 -80 -60 -40

HCN2

membrane potential (mV)

-140 -120 -100 -80 -60 -40

instantaneous (pA)

-140 -120 -100 -80 -60 -40

B

HCN1

input conductance (nS)

0 0.4 0.8 1.2 1.6

control propofol cesium

* *

HCN1-HCN2

input conductance (nS)

0 0.4 0.8 1.2 1.6

control propofol cesium

* *

HCN2

input conductance (nS)

0 0.4 0.8 1.2 1.6

control propofol cesium

*
bicuculline (30 μM) + strychnine (30 μM)
propofol (5 μM)

Figure 6
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