Propofol-Block of SK Channels in Reticular Thalamic Neurons Enhances GABAergic Inhibition in Relay Neurons

Shui-Wang Ying and Peter A. Goldstein
C. V. Starr Laboratory for Molecular Neuropharmacology, Department of Anesthesiology, Weill Medical College, Cornell University, New York, New York

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Ying, Shui-Wang and Peter A. Goldstein. Propofol-block of SK channels in reticular thalamic neurons enhances GABAergic inhibition in relay neurons. J Neurophysiol 93: 1935–1948, 2005. First published November 24, 2004; doi:10.1152/jn.01058.2004. The GABAergic reticular thalamic nucleus (RTN) is a major source of inhibition for thalamocortical neurons in the ventrobasal complex (VB). Thalamic circuits are thought to be an important anatomic target for general anesthetics. We investigated presynaptic actions of the intravenous anesthetic propofol in RTN neurons, using RTN-retained and RTN-removed brain slices. In RTN-retained slices, focal and bath application of propofol increased intrinsic excitability, temporal summation, and spike firing rate in RTN neurons. Propofol-induced activation was associated with suppression of medium afterhyperpolarization potentials. This activation was mimicked and completely occluded by the small conductance calcium-activated potassium (SK) channel blocker apamin, indicating that propofol could enhance RTN excitability by blocking SK channels. Propofol increased GABAergic transmission at RTN-VB synapses, consistent with excitation of presynaptic RTN neurons. Stimulation of RTN resulted in synaptic inhibition in postsynaptic neurons in VB, and this inhibition was potentiated by propofol in a concentration-dependent manner. Removal of RTN resulted in a dramatic reduction of both spontaneous postsynaptic inhibitory current frequency and propofol-mediated inhibition of VB neurons. Thus the existence and activation of RTN input were essential for propofol to elicit thalamocortical suppression; such suppression resulted from shunting through the postsynaptic GABAA receptor-mediated chloride conductance. The results indicate that propofol enhancement of RTN-mediated inhibitory input via blockade of SK channels may play a critical role in “gating” spike firing in thalamocortical relay neurons.

INTRODUCTION

The thalamic reticular nucleus (RTN) is a thin sheet of GABAergic neurons that surrounds the anterior, lateral surfaces of the thalamus and receives excitatory input from collaterals of both corticothalamic and thalamocortical axons (Jones 1985; Ohara and Lieberman 1985). RTN provides a major source of inhibitory input to thalamocortical neurons in the ventrobasal complex (VB) in rodents (Liu et al. 1995). Since VB lacks GABAergic interneurons, GABAergic inhibitory control of VB neuron responsiveness to excitatory inputs is primarily mediated through feed-forward inhibition (cortex-RTN-VB), and feedback inhibition (RTN-VB; Sherman and Guillery 2001; Steriade et al. 1997). Through GABAergic inhibition, RTN has been implicated in several important functions, including influencing the efficacy of sensory input (Le Masson et al. 2002; Lee et al. 1994), altering thalamic relay responses to corticothalamic excitatory input (Ergenzinger et al. 1998; Hartings and Simons 2000; Temereanca and Simons 2004), gating selective attention (Guillery et al. 1998; Hartings et al. 2000, 2003; Weese et al. 1999), synchronizing thalamic oscillations (Steriade et al. 1997), and generating sleep spindles and slow-wave sleep (Steriade 2001). Recent evidence has indicated that, through RTN-mediated intra-thalamic connections, intra-thalamic nuclei (e.g., VB, posterior complex, ventrolateral nucleus, and intralaminar nuclei) can interact with each other, and this circuitry enables RTN to play a pivotal role in switching between waking and sleep states and regulating thalamic sensory and motor information processing (Crabtree 1999; Crabtree and Isaac 2002; Crabtree et al. 1998).

Propofol is an intravenous anesthetic with a chemical structure distinct from any other anesthetic and is also a potent modulator of GABAA receptors (Trapani et al. 2000). Propofol has been shown to potentiate GABAergic responses in postsynaptic neurons in several brain regions (Bai et al. 1999; Bieda and Maclver 2004; Chen et al. 1999; Collins 1988; Inoue et al. 1999; Jurd et al. 2003; Kitamura et al. 2003; Manuel and Davis 1998; Peduto et al. 1991; Reynolds et al. 2003; Wakesugi et al. 1999). Although effects of GABAergic anesthetics including propofol are generally thought to be mediated by GABAergic mechanisms, such a concept is mainly based on the assessment of anesthetic effects on GABAergic currents in postsynaptic neurons or non-neuronal systems. The manner in which propofol directly influences presynaptic GABAergic neurons is largely unknown. Therefore this study investigated the effects of propofol on presynaptic GABAergic RTN neurons using mouse brain slices. To assess the role of RTN in mediating anesthetic actions in the thalamus, we studied the ability of propofol to influence VB neuron spike firing in RTN-retained and RTN-removed slices. Preliminary results have been published in abstract form (Ying and Goldstein 2002).

METHODS

Brain slice preparation

Experiments were performed in accordance with institutional and federal guidelines. Mice (C57BL/6, P25-55) were anesthetized by halothane and decapitated. The head was immediately submerged in ice-cold carbogenated (95% O2-5% CO2) slicing solution, and the brain was rapidly dissected out. To prepare horizontal slices, the brain was sagittally cut into two halves along the midline; slices (240 or 300 μm) were horizontally cut on a microslicer (VT 1000S, Leica, Wetzlar, Germany) using a sapphire blade (Leica) to yield smooth-
surface slices. Thalamocortical (TC) slices were prepared as described (Agmon and Connors 1991) with a slight modification. Briefly, after the brain was dissected out, the rostral portion of the brain was cut at 45 or 55°; the rostral end of the brain block was glued to a homemade platform, and slices (300 μm) were cut. Slices were gently rinsed once in cold artificial cerebrospinal fluid (ACSF) and incubated in carbogenated ACSF at 34°C for 1 h for recovery and then at 24°C for at least another 1 h before use. Horizontal slices were used in most experiments, and TC slices were used for recording of spontaneous activity in VB and for corticothalamic pathway stimulation. All slices had intact RTN connections, unless otherwise specified (see RESULTS).

**Electrophysiology**

Current-clamp recordings were performed at 35°C. Slices were perfused with carbogenated ACSF; neurons were visualized and identified using a Zeiss Axioskop (Jena, Germany) equipped with a ×2.5 objective and ×40 water immersion objective with a 2.4-mm working distance and IR-DIC optics. Recordings were made from neurons located in either the RTN or VB; all RTN neurons were located in either somatosensory sector or the anterior RTN (Cox et al. 1996; Guillery et al. 1998; Ohara and Lieberman 1985). The resistance of the pipette was 3.5–6.0 MΩ when filled with internal solution. Tight seal (>2. GΩ) was achieved by application of a small negative pressure, using a 1-ml syringe. Access resistance (Rₐ) was compensated by ≤70% and ranged from 10 to 14 MΩ after compensation; data were discarded if Rₐ > 15 MΩ. Steady-state input resistance was measured at a holding membrane potential level close to resting membrane potential (RMP) from the voltage response elicited by a small current pulse (−60 pA, 500 ms). Only neurons that showed a stable RMP negative to >60 mV, action potential overshoot of >10 mV, and input resistance >150 MΩ were selected for study. Although cells so selected generally showed stable data records for ≥240 min, pharmacological tests were completed within 90 min to minimize the variation of responses. Liquid junction potentials (11.1–12.2 mV) were calculated, based on intracellular and bath solutions, using the software Junction Potential Calculator (Clampex 9, Axon Instruments, Union City, CA) and corrected on-line or off-line. Voltage-clamp recordings of inhibitory postsynaptic currents (IPSCs) were made at 25°C, using a Cs⁺-based internal solution (Goldstein et al. 2002). Neurons were voltage-clamped at a holding potential of −60 mV, except for those experiments in which various holding potentials were used to test the effect of electromotive driving force on miniature IPSC (mIPSC) amplitude. Membrane current was low-pass filtered at 2 Hz, and membrane voltage was low-pass filtered at 5 kHz and digitized at 10 kHz using an Axopatch 200A amplifier connected to a DigiData 1200 interface (Axon Instruments). In some experiments, a Multiclamp 700B amplifier connected to a DigiData 1322A interface (Axon Instruments) was used. Data were collected ≥10 min after whole cell access to allow the pipette solution to equilibrate with the neuron.

**Extracellular and intracellular electrical stimulation**

To evoke synaptic responses in RTN neurons, a concentric bipolar tungsten electrode (FHC, Bowdoinham, ME) was placed in either layer VI of the barrel cortex or the white matter in TC slices (Pedrocchina and Llinás 2001). To evoke inhibitory postsynaptic potentials (IPSPs) or IPSCs in VB neurons, the stimulation electrode was placed in RTN (at somatosensory sector) or the internal capsule (Warren et al. 1997). Single pulses were delivered using a Master-8 pulse generator (A.M.P.I., Jerusalem, Israel) controlled by a PC, and stimulation intensity was controlled by a constant current stimulus isolator (World Precision Instruments, Sarasota, FL). Responses were considered monosynaptic if the latency jitter was <0.4 ms and their rise times were consistent from trial to trial (3 trials). To confirm that responses were GABA_A receptor mediated, a GABA_A receptor antagonist (10 μM bicuculline or gabazine) was used to block responses in the presence of a GABA_A receptor antagonist (100 μM 2-0H saclofen or 1 μM CGP55845). To identify excitatory synaptic potentials (EPSPs), CNQX (20 μM) and n-AP5 (40 μM) were used to block evoked EPSPs, and the Na⁺ channel blocker TTX (500 nM) was used to block evoked action potentials. Intrinsic spike firing was initiated with intracellular DC injection or rectangular current pulses (500–1,000 ms). To study temporal summation of intrinsic subthreshold-old voltage responses at the soma, five depolarizing exponential (EPSP-shaped) current pulses were generated with the function: f(t) = A × (1 – exp(−(t/τrise) × exp(−(t/τdecay)))), where A is the amplitude of the current (200–300 pA), n (=5) is an integer, and τrise and τdecay are rising (2 ms) and falling (20 ms) time constants, respectively (Magee 1998).

**Bath and focal application of drugs**

Drugs were generally applied by bath superfusion (unless otherwise noted) for ≥20 min at a flow rate of 3 ml/min prior to data collection. The perfusion system consisted of polytetrafluoroethylene (Teflon) tubing and connectors. Propofol was freshly prepared in DMSO and diluted with ACSF to 0.3–6 μM, which was within clinically relevant concentration ranges (Franks and Lieb 1998). The final concentration of DMSO was 0.1%, which had no effects on the cells examined. In some cases, a drug solution was focally ejected onto the soma through a puffer pipette connected to Picospritzer II (Parker Instruments, Fairfield, NJ) using a pico-ejection technique. The pipette tip was placed 30–40 μm away from and ~20 μm below the soma, and specific responses to a drug were verified by a corresponding antagonist applied by bath whenever possible. The pipette tip was checked after use to see whether it was clogged.

**Solutions**

Slicing solution contained (in mM) 2.5 KCl, 24 NaHCO₃, 1.25 NaH₂PO₄, 234 sucrose, 11 glucose, 10 MgSO₄, and 0.5 CaCl₂. ACSF (bath solution) for current-clamp recordings contained (in mM) 124 NaCl, 26 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 1.2 MgCl₂, 2 CaCl₂, and 11 glucose. Intracellular solution contained (in mM) 130 K-glucate, 5 NaCl, 2 MgCl₂, 10 HEPES, 0.5 EGTA, 2 ATP-K, and 0.3 GTP- Na, pH adjusted to 7.25 with KOH. K-glucate was used because the impermanent ion gluconate does not contribute to anesthetic-induced changes in RMP or I-V relationship (Nishikawa and MacIver 2000). Bath solutions were freshly prepared on the same experimental day. The bath solution for voltage-clamp contained (in mM) 117 NaCl, 25 NaHCO₃, 3.6 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 2.5 CaCl₂, and 11 glucose; osmolality was adjusted to 300 mOsm with sucrose.

**Chemicals**

Propofol was from Aldrich (Milwaukee, WI) or TCI (Portland, OR). (+)-Bicuculline, gabazine, 2-0H saclofen, (2S)-3-[(1S)-1-(3,4-dichlorophenyl) ethyl] amino-2-hydroxypropyl] (phenylmethyl) phosphinic acid (CGP55845), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and D-2-amino-5-phosphopentanoic acid (D-AP5) were from Tocris Cookson (Ellisville, MO). TTX was from Alomone Labs (Jerusalem, Israel).

**Intracellular biocytin filling**

Neurons from 30 mice were intracellularly filled with biocytin (0.5% in the pipette solution) to verify recording sites. After recording, slices were fixed for 24–72 h in phosphate buffer (PB) solution containing 4% paraformaldehyde, transferred to 20% sucrose solution in 0.1 M PB, and resectioned to 60–100 μm. After endogenous peroxidases were blocked with phosphate-buffered 3% H₂O₂, the slices were incubated with biotinylated horseradish peroxidase con-
jugated to avidin (ABC-Elite, Vector Labs, Burlingame, CA), washed, and incubated with DAB for 15 min. Filled neurons were visualized using IR-DIC optics and drawn as previously described (Kawaguchi 1993).

Data and statistical analysis

Data were analyzed using both Clampfit 9 and MiniAnalysis 6.0.4. To analyze temporal summation containing five responses, the peak of the first and fifth responses was measured from baseline, and expressed as \( \Delta V \) and \( \Delta V_s \), respectively; responses were calculated as \( \% \text{increase} = \left( \frac{\Delta V_s}{\Delta V} - 1 \right) \times 100 \). Temporal summation was defined as percent increase in depolarization occurring at the soma during a train (Magee 1999). Action potential parameters including amplitude, threshold, duration, and rise/decay time were also obtained. For analysis of fast afterhyperpolarization (fAHP) followed by a single spike, 100–300 successive spikes were averaged, and fAHP peak amplitude was measured from the baseline to the maximum negative point (peak). For analysis of medium-duration AHP (mAHP), mAHP was integrated (AHP area), between the point at which the spike falling phase reached the baseline and the point that the negative voltage deflection returned to the baseline (Sourdut et al. 2003). For percent spike firing suppression in experiments with RTN stimulation, the average number of spikes was obtained from at least three trials; percent inhibition was calculated as \( (F2/F1 - 1) \times 100 \), where \( F1 \) and \( F2 \) were the average number of spikes recorded before and during treatment (drug application or electrical stimulation), respectively. To study GABAergic synaptic transmission at RTN-VB synapses, stimulation intensity was set to evoke IPSCs in ~50% of the stimulations, as calculated from 30 stimuli (3 trials, each containing 10 sweeps), and evoked responses with deflections from baseline <4 \times \text{RMS noise} were considered failures (Liu et al. 2001). The concentration-effect curves were fitted with the Hill equation: \( y = \frac{V_{\text{max}}}{1 + \left( \frac{x}{k} \right)^n} \), where \( y \) is percent inhibition at the drug concentration \( x \), \( V_{\text{max}} \) is the maximal percent inhibition, \( k \) is the EC_{50} value (concentration needed for a half-maximum response), and \( n \) is the Hill slope constant. Statistical analyses were performed with Sigmastat V3.0 (SPSS, Chicago, IL) using t-test or one-way ANOVA with post hoc test where appropriate. Large IPSCs (presumed action potential-dependent events) were defined as IPSCs with amplitude >240 pA, since no IPSCs greater than this value were observed in the presence of the sodium channel blocker TTX (500 nM) under our experimental conditions. Cumulative amplitude distributions for IPSCs were generated, and differences in amplitude distributions were tested by Kolmogorov-Smirnov test (MiniAnalysis V6.0.4), with a significant difference at \( P < 0.05 \). Data are expressed as mean ± SE.

RESULTS

Neurons in RTN were readily located and identified in live brain slices using IR-DIC optics. In a number of instances, the position of the recorded neuron was confirmed using biocytin labeling (data not shown). The distinct membrane properties of GABAergic RTN and glutamatergic VB neurons allowed us to identify the two types of neurons, as shown in Fig. 1A. In response to a hyperpolarizing current pulse, RTN neurons showed a very shallow depolarizing sag regardless of membrane potential level; they generally showed multiple, rhythmic rebound spike bursts at relatively hyperpolarized membrane potentials, with no slow after-burst depolarization potential (ADP). All these features were identical to previous observations (Debarbieux et al. 1998; reviewed by Destexhe and Sejnowski 2001), and the rebound burst pattern was consistent with that observed for type I neurons in the cat RTN in vivo (Contreras et al. 1992). In contrast, VB neurons displayed a

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FIG. 1. Propofol increases intrinsic excitability and spike firing in reticular thalamic nucleus (RTN) neurons. A: distinct voltage responses in RTN and ventrobasal complex (VB) neurons. In response to a hyperpolarizing current pulse (~500, 500 ms), an RTN neuron generally showed a shallow sag, with multiple rebound spike bursts and without after-burst depolarization potential (ADP) compared with prominent sag and ADP in a VB neuron. B: tonic spike firing was initiated in a different neuron with DC injection, and bath application of propofol (3 μM) increased spike firing rate (Bi). Bii: effects of propofol on tonic spike firing rate. *P < 0.05, 1-way ANOVA vs. control, n = 18. C: intrinsic spike firing was initiated by intracellular current pulse injection (~400 and 100 pA, 500 ms). Ci: focal propofol (3 μM in pipette) increased tonic and rebound spike firing. Focal drug application was started 10 s prior to recordings. Cii: small voltage responses to a hyperpolarizing current pulse (~60 pA, 500 ms) in the absence and presence of propofol. Note that propofol increased input resistance. Ciii: effects of propofol on input resistance. *P < 0.05, 1-way ANOVA vs. control, n = 20. Amplitude scale: 50 mV for Ci and Ciii; 10 mV for Ciiii. Value to the left of each voltage trace in this and following figures indicates the membrane potential.
large, slow depolarizing sag and slow ADP in response to the same protocol (Fig. 1A bottom, protocol not shown).

**Propofol increases intrinsic excitability and spike firing in RTN neurons**

Propofol has been shown to produce a sedative effect, accompanied by an increase in GABAergic neuronal activity in the ventrolateral preoptic nucleus, a component of a sleep-promoting pathway in vivo (Nelson et al. 2002). Here we investigated effects of propofol on intrinsic excitability in GABAergic RTN neurons. Tonic spike firing was initiated with a depolarizing DC (Fig. 1Bi). Bath application of propofol increased the spike firing rate; an example trace is shown in Fig. 1Bii. Group data (n = 18) showed that propofol increased RTN spike firing by 28 ± 4 and 72.8 ± 8% at concentrations of 0.6 and 3 μM, respectively (Fig. 1Biii). To test for a direct effect in RTN, propofol (3 μM in pipette) was focally applied to the soma through a 1-Ω glass pipette. Focal application produced a similar effect (n = 5, data not shown).

To study how propofol affected both spike firing patterns and input resistance that is associated with intrinsic excitability, intrinsic spike firing was initiated with depolarizing and hyperpolarizing current pulses. RTN neurons showed both tonic spike firing and rebound spike bursts in response to current pulse injections (Fig. 1Ci). The intrinsic voltage response was consistent with that observed for type I neurons in the cat RTN in vivo (Contreras et al. 1992). Input resistance was 320 ± 18 Ω (n = 20) in control; focal application of propofol (0.6 and 3 μM) increased input resistance to 390 ± 12 and 429 ± 17 Ω, respectively, accompanied with an increase in single spike firing and rebound spike bursts (Fig. 1Cii). The activating effects produced by bath and focal application of propofol were similar, and data were therefore pooled. Normalized group data for input resistance (n = 20) are shown in Fig. 1Civ.

**Propofol suppresses apamin-sensitive mAHP**

The above data raised the question of how propofol increased intrinsic excitability. When RTN neurons fired single spikes, the spikes were followed by fAHPs (lasting 1–2 ms) and mAHPs (lasting ~300 ms). Analyzing spikes in another 20 RTN neurons activated by focal propofol, we found that mAHP was suppressed (Fig. 2A). Comparison of differences in AHPs in the absence (control) and presence of propofol is shown in Fig. 2B. Other spike parameters, including the threshold for spike firing and fAHP amplitude, were not significantly changed.

As reported for hippocampal neurons, mAHP is mediated by small-conductance Ca²⁺-activated potassium channels (SK channels) that regulate tonic firing rate, and mAHP is

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**FIG. 2.** Propofol suppresses medium afterhyperpolarizations (AHPs) but not fast AHPs in RTN neurons. A: tonic spike firing was recorded from a RTN neuron (control). Focal application of propofol (3 μM in pipette) increased the firing frequency. B: comparison of the fast AHP (fAHP; left) and medium AHP (mAHP; right) in the absence (control) and presence of propofol. Note that propofol markedly reduced mAHP but not fAHP (same neuron as in A). Each trace is an average of 500 successive spikes, and spikes are truncated to 50 ms. Time scale: left, 5 ms; right, 100 ms. C: tonic spike firing was recorded from another RTN neuron (control). Focal application of the SK channel blocker apamin (200 nM in pipette) increased spike firing (middle), and addition of propofol (by bath) had no further effect (right). D: overlay of traces showing fAHP (Di) and mAHP (Dii) in the absence (control) and presence of apamin. Note that apamin markedly reduced mAHP but not fAHP. Dii: overlay of traces showing mAHP in the presence of apamin alone or apamin plus propofol. Note that apamin completely occluded the effect of propofol on mAHP. Each trace is an average of 500 successive spikes. Time scale: Di; 2 ms; Dii; 100 ms. E: bar graph summarizing the effects of propofol and apamin on mAHPs. *P < 0.05 vs. control, 1-way ANOVA with Tukey pair-wise comparisons, n = 15. F: effects of apamin and propofol on spike firing; *P < 0.05 vs. control, n = 14, the same statistical analyses here (and in G) as in E. G: effect of apamin and apamin plus propofol on input resistance; pooled data indicate that apamin increased input resistance. *P < 0.05 vs. control. There was no significant difference in effects between propofol and apamin plus propofol or apamin and propofol plus propofol (E–G), indicating that apamin occluded effects of propofol on mAHP, spike firing, and input resistance.
highly sensitive to the SK channel blocker apamin (Stocker et al. 1999). To investigate if the apamin-sensitive mAHP was involved in regulating responses to propofol, apamin (200 nM in pipette) was focally applied to the soma in another subgroup of RTN neurons. Apamin reduced mAHP and increased both input resistance and tonic spike firing (Fig. 2, C–G). As shown in Fig. 2D, apamin decreased mAHP in a manner similar to propofol. The addition of propofol (3 µM) during apamin application or co-application of the two compounds produced a similar voltage response (Fig. 2, C and D). Group data are shown in Fig. 2, E–G. Both propofol and apamin markedly decreased mAHP and increased spike firing and input resistance in a similar manner; there was no significant difference in effects produced by apamin alone and apamin plus propofol groups. This clearly showed that the effect of propofol was mimicked and completely occluded by apamin. Thus the increase in RTN neuron intrinsic excitability, and tonic spike firing during propofol application was primarily due to blockade of the apamin-sensitive SK channel. 

**Propofol increases temporal summation in RTN neurons independently of excitatory synaptic input**

The above data indicated that propofol markedly increased input resistance in RTN neurons. Alterations in input resistance are known to critically influence temporal summation of subthreshold voltage responses and, in turn, action potential generation (Magee 1998). Whether propofol was capable of affecting subthreshold responses via intrinsic excitability was directly investigated; intrinsic voltage responses (in the absence of major synaptic inputs) were initiated with intracellular injection of EPSC-shaped current pulses (train of 5 pulses, 33Hz), as done for hippocampal neurons (Magee 1998). Under control conditions (no drug), we found that the intrinsic subthreshold voltage response was incrementally increased during a train (Fig. 3Ai), with an increase in summation of 32.2 ± 4.1% (n = 15). Focal application of propofol (3 µM in pipette) increased the summation (Fig. 3Aii). A clear comparison of differences in summation prior to and during propofol application is shown in Fig. 3Aiii. Group data (Fig. 3Avi) showed

![Fig. 3. Propofol enhances temporal summation of intrinsic subthreshold voltage responses via blockade of small conductance calcium-activated potassium (SK) channels in RTN neurons. Ai: intrinsic subthreshold voltage response was initiated in a RTN neuron with intracellular injection of an excitatory postsynaptic current (EPSC)-shaped current train (33 Hz, 5 pulses, 200 pA, bottom). Aii: focal application of propofol (3 µM in pipette) enhanced temporal summation. Aiii: overlay of i and ii for comparison (1st and 5th responses indicated by arrows). Avi: propofol enhanced summation of intrinsic subthreshold responses in RTN neurons. *P < 0.001, t-test, n = 15. Bi: in a different RTN neuron, the intrinsic voltage response was initiated with the same current pulse train as in Ai at more depolarized membrane potential. Bii: bath application of propofol increased the number of spikes. Ci: in another RTN neuron, focal application of apamin (200 nM in pipette) mimicked the effect of propofol. Ciii: addition of propofol failed to alter spike generation, indicating that apamin occluded propofol’s effect.](http://jn.physiology.org/)

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that propofol significantly increased summation of the intrinsic response (56.8 ± 5%, \( P < 0.001, t\)-test). Detailed analyses of integrative properties are summarized in Table 1.

In another subgroup of RTN neurons (\( n = 5 \)), intrinsic voltage responses were initiated with the same intracellular EPSC-shaped pulses at more depolarized membrane potential (−60 to −58 mV). As shown in Fig. 3Bi, summation was associated with the occurrence of a single spike, and bath application of propofol increased the number of spikes to six (Fig. 3Bii). To examine whether the SK channel–mediated conductance might be involved, the same response (control) was initiated in another RTN neuron (Fig. 3Ci). Focal application of apamin (200 nM in pipette) resulted in a response identical to propofol (Fig. 3Cii); subsequent bath application of propofol had little effect on summation (Fig. 3Ciii). These data indicated that propofol could enhance intrinsic subthreshold responses in RTN neurons via blockade of the apamin-sensitive SK channel and that this enhancement was independent of excitative synaptic input.

As shown in Figs. 1, 3, and 4, propofol depolarized RTN neurons. Group data showed that propofol depolarized the membrane potential by 2.6 ± 0.5 mV, and this change, although modest, was significant (Wilcoxon signed-rank test, \( P < 0.001, n = 68 \)). The SK channel blocker apamin produced a nearly identical depolarizing effect on the membrane potential (\( P < 0.001, n = 24 \), data from Figs. 2–4). Thus the depolarization during propofol application appeared due to blockade of SK channels, consistent with other observations (Cingolani et al. 2002; Hallsworth et al. 2003).

**Propofol can enhance synaptic temporal summation through the suppression of SK channels**

RTN receives massive excitatory synaptic input from the cortex (Steriade et al. 1997). Increased intrinsic excitability in thalamic neurons can enhance synaptic responsiveness to corticothalamic excitatory input (Pedroarena and Llinás 2001). We hypothesized, therefore, that propofol-mediated enhancement of intrinsic excitability might also alter synaptic temporal summation. Effects of propofol on EPSP summation in RTN neurons were investigated by extracellular stimulation of corticothalamic fibers (5 pulses, 33 Hz) in thalamocortical slices. We found that corticothalamic stimulation in the absence of drug consistently gave rise to EPSP summation (602%) at membrane potentials of −66 mV (Fig. 4Ai). Bath application of propofol (3 \( \mu M \)) depolarized the membrane potential by 3 mV and increased synaptic temporal summation to 870% (Fig. 4Ai). Differences in synaptic summation in the absence (con-
control) and presence of propofol are shown in Fig. 4Aiii. Group data (Fig. 4Aiv) clearly indicated that propofol significantly increased synaptic temporal summation in RTN neurons ($P < 0.001, t$-test, $n = 15$). The effects of propofol on integrative properties of synaptic responses are summarized in Table 1.

We next examined whether propofol could alter synaptic responses during SK channel blockade for at least three reasons. First, the SK channel–mediated conductance can contribute to synaptic efficacy by altering neuronal excitability in postsynaptic neurons (Borde et al. 1999). Second, excitatory input from the cortex persists, albeit reduced, during propofol application at concentrations of 0.3–3 μM (unpublished observations; see also Antkowiak 1999). Third, propofol does not influence glutamatergic excitatory transmission (Bickler et al. 1995; Kitamura et al. 2003); this last point enabled us to simplify the analysis of the possible mechanisms involved.

Synaptic summation was evoked at relatively depolarized membrane potentials, so that repetitive synaptic stimuli could summate to fire single spikes, and low threshold conductances could be minimized (Pedroarena and Llinás 2001). Five synaptic stimuli evoked a single spike at the fifth stimulus when the membrane potential was held at −61 mV in control (Fig. 4Bi); bath application of propofol (3 μM) increased the number of evoked spikes (Fig. 4Bii). Similar synaptic responses were evoked in another neuron (Fig. 4Ci), and focal application of apamin (200 nM) produced a response identical to that elicited by propofol (Fig. 4Cii); subsequent addition of propofol produced a similar response (Fig. 4Dii). The same responses were observed in five of five neurons. Again, this indicated that the effect of propofol was occluded by apamin and that blockade of the SK channel during propofol application could enhance synaptic responses in RTN neurons.

**Propofol enhances successful GABAergic transmission at RTN-VB synapses**

As shown above, propofol enhanced GABAergic RTN neuronal excitability and spike firing; we predicted that there should be a concomitant increase in GABAergic input to VB, and this possibility was directly investigated using voltage-clamp recordings in VB neurons. We first examined whether the change of RTN excitability could alter the success rate of GABAergic synaptic transmission from RTN to VB (Ulrich and Huguenard 1995). GABA$_A$ IPSCs were evoked in VB neurons clamped at −60 mV in the presence of 2-OH saclofen (100 μM) by extracellular stimulation of RTN, with stimulus intensity (20–100 μA, 0.15 ms, every 10 s) set to evoke EPSCs in ~50% of the stimulations in control (Mansvelder et al. 2002). Evoked fast unitary IPSCs (eIPSCs) had an average latency of 2.4 ± 0.8 ms (from time of stimulus to onset of IPSC). As shown in Fig. 5Ai, 10 synaptic stimuli evoked an average of five IPSCs (averaged from 3 trials, with 10 stimuli/each). Bath application of propofol (0.6 μM) increased the average number of evoked IPSCs to eight (Fig. 5Aii) in the same neuron (using the same stimulation protocol). Normalized group data (Fig. 5Aiii) showed that propofol increased the success rate of GABAergic transmissions at RTN-VB synapses in a concentration-dependent manner, strongly indicating a presynaptic site of action (Ulrich and Huguenard 1995).

Presynaptic mechanisms underlying the effect of propofol on GABAergic transmission may involve modulation of transmitter release, which is reflected by a change in spontaneous IPSC (sIPSC) frequency (Richards 2002). Spontaneous IPSCs (also termed “total” IPSCs) include action potential–dependent and –independent events; the two IPSC types can be distinguished by the use of TTX (Nishikawa and Maclver 2000; Semyanov and Kullmann 2001). Spontaneous IPSCs were recorded in VB neurons at a holding potential of −60 mV in the absence of TTX (Fig. 5Bi), and bath application of propofol (0.6 μM) increased the frequency of total IPSCs (Fig. 5B); 3 μM propofol produced a larger effect (traces not shown). Analysis of pooled data showed that there was a significant difference in the frequency of total IPSCs before and during propofol application at 0.6 and 3 μM (Fig. 5Biv, $P < 0.05$, 1-way ANOVA, vs. control, $n = 15$).

Action potential–dependent IPSCs have been successfully used to study GABA release due to presynaptic spike firing (Dumoulin et al. 2001; Nishikawa and Maclver 2000) rather than the release machinery located in nerve terminals (Dunlap et al. 1995; Jarvis and Zamponi 2001; Richards 2002). Presumed action potential–dependent IPSCs were defined as IPSCs > 240 pA (also termed large IPSCs), because under our experimental conditions, no IPSC greater than this value was observed after application of TTX (500 nM), which completely abolished action potential firing (not shown). Propofol at 0.6 and 3 μM markedly increased the large IPSC frequency ($P < 0.05$, 1-way ANOVA, vs. control, Fig. 5Ci), but did not significantly increase large IPSC amplitude (Fig. 5Cii). Therefore the data showed that increasing RTN neuronal excitability was coupled to an increase in spontaneous IPSC frequency.

**Effects of propofol on miniature IPSCs**

Some anesthetics may alter GABA release by directly modifying the release machinery in nerve terminals (reviewed by Richards 2002). Such an effect can manifest itself by changing mIPSC frequency, and this possibility was also tested in another subgroup of VB neurons. mIPSCs were recorded in VB neurons in the presence of TTX (500 nM) at a holding potential of −60 mV and had an average amplitude of 43.2 ± 2 pA (n = 15; Fig. 6Ai). Bath application of propofol significantly increased mIPSC amplitude by 36.1 ± 3.3% at 0.6 μM and 44.7 ± 3.2% at 3 μM, respectively ($P < 0.05$, 1-way ANOVA, vs. control, $n = 15$, Fig. 6Bi, traces for 0.6 μM not shown). At the lower concentration (0.6 μM), propofol had little effect on mIPSC frequency. At the higher concentration (3 μM), however, propofol significantly increased frequency (19 ± 1.1%, from 13.7 ± 1.2 to 16.3 ± 1.4 Hz, $P < 0.05$, 1-way ANOVA, vs. control, Fig. 6Bii). Since mIPSC amplitude was markedly increased by 3 μM propofol, a moderate increase in the frequency might be due to an increase in event detection. To directly test to what extent mIPSC amplitude could affect the event detection probability, holding potentials were set to more positive levels so as to increase amplitude by a magnitude similar to that produced by propofol. At a holding potential of −50 mV (no propofol), mIPSC amplitude increased by 27.1 ± 2.2% ($P < 0.05$ compared with mIPSC amplitude recorded at −60 mV), but frequency did not change. At a holding potential of −40 mV, mIPSC amplitude was further increased (by 45.5 ± 3.5% compared with mIPSC amplitude recorded at −60 mV), and mIPSC frequency was significantly increased.
The increase in mIPSC amplitude produced by the more positive holding potential (−40 mV) was essentially identical to that produced by 3 μM propofol (45.4 and 44.7%, respectively). This indicated that a large increase in mIPSC amplitude could increase the probability of event detection and that 3 μM propofol might not directly modulate GABAergic terminals as to increase mIPSC frequency.

**Propofol enhanced GABA<sub>A</sub> synaptic inhibition in thalamocortical neurons**

Clinical studies have shown that propofol-induced unconsciousness is accompanied by thalamic inhibition of somatosensory-evoked responses in humans (Bonhomme et al. 2001). This inhibition may result from anesthetic potentiation of RTN-mediated inhibitory input to thalamocortical neurons. Therefore the effect of propofol on synaptic inhibition of VB neuron spike firing was investigated. Tonic spike firing was initiated prior to stimulation (Fig. 7Ai). GABA<sub>A</sub> IPSPs were evoked by extracellular single stimuli (50–100 μA, 0.15 ms, every 10 s) in the presence of 2-OH saclofen (100 μM). RTN stimulation produced a small IPSP (~5 mV in amplitude, ~60 ms in duration) and decreased spike firing (Fig. 7Aii). Evoked IPSPs and inhibition of spike firing could be reversibly blocked by the specific GABA<sub>A</sub> receptor antagonist gabazine (10 μM, Fig. 7Aiii). Bath application of propofol (0.6 μM; right) increased the duration (~106 ms), thus enhancing the synaptic inhibition (Fig. 7Aiv). The propofol-potentiated IPSP and enhanced inhibition of spike firing could be blocked by gabazine (Fig. 7Aivi).

FIG. 5. Propofol enhances GABAergic transmission at RTN-VB synapses. Ai: inhibitory postsynaptic currents (IPSCs) were evoked in a VB neuron clamped at −60 mV by RTN stimulation with stimulus intensity (20 μA; 100, 150 μs, every 15 s) set to produce ~50% success rate in control (left). Aii: bath application of propofol (0.6 μM; right) increased the number of synaptically evoked IPSCs in the same cell as in Ai. Aiii: propofol enhanced success rate of synaptic transmission in a concentration-dependent manner. *P < 0.05 vs. control, 1-way ANOVA, n = 15. Bi: spontaneous IPSCs were recorded in another VB neuron at −60 mV in a TC slice. Bii: propofol (0.6 μM) increased IPSC frequency. Note a marked increase in large-amplitude IPSC frequency. Biii: time course plot for pooled data (n = 15) showing an increase in total sIPSC frequency in the presence of propofol. Biv: effect of propofol on total IPSC frequency at 2 different concentrations. *P < 0.05 vs. control (0 μM), n = 15. Ci: propofol significantly increased the frequency of large IPSCs. *P < 0.05 vs. control. Cii: propofol had no significant effect on the amplitude of large IPSCs (same neurons as in Biv).
mIPSC amplitude and frequency; note that propofol at 0.6 mM increased mIPSC frequency, and prolonged the decay time. *P < 0.05 vs. control (0 mM), n = 15. Ii and Iii: effect of propofol on mIPSC amplitude and frequency; note that propofol at 0.6 mM does not increase mIPSC frequency. *P < 0.05 vs. control (0 mM), n = 15. Ci and Cii: effect of holding potentials on mIPSC amplitude and frequency. *P < 0.05 vs. mean control (−60 mV).

mean reversal potential of −71.1 and −70.7 mV in the control and propofol groups, respectively (Fig. 7Ciii). The values were close to calculated reversal potential of −69 mV for Cl− (based on solutions for current clamp), and there was no significant difference in the mean reversal potential before and during propofol application. Propofol failed to alter the reversal potential or to trigger spike bursting from depolarizing IPs.

RTN input is critical in mediating propofol’s effects in VB

Next, we investigated the extent to which RTN-mediated inhibitory input influenced spike firing in VB neurons during propofol application. RTN is the sole source of GABAergic input to VB because the rodent VB does not contain GABAergic interneurons (Arcelli et al. 1997). In horizontally cut brain slices, RTN looks like a narrow band, lying anteriorly and laterally to VB, and a large part of the anterior (somatosensory sector) RTN projects to VB (Cox et al. 1996; Ohara and Lieberman 1985). This unique structure enabled us to minimize GABAergic input to VB by excising RTN and neighboring structures (e.g., the striatum, cortex, and other structures, see Fig. 8A), thereby creating “RTN-removed” slices. “RTN-retained” slices were prepared from the contralateral hemisphere at an identical plane and served as controls.

To test the extent to which GABAergic input to VB was altered in RTN-removed slices, we recorded sIPSCs and analyzed sIPSC frequency. In RTN-retained slices, there were robust sIPSCs (Fig. 8B, top), with a frequency of 18.2 ± 1.8 Hz and amplitude of 8–1,500 pA. In RTN-removed slices, spontaneous IPSC frequency was reduced to 4.5 ± 1.2 Hz, and amplitude was much smaller (7–80 pA; Fig. 8C). The differences in sIPSC frequency and amplitude between RTN-removal and RTN-retained slices were significant (P < 0.001, t-test, n = 12). All IPs could be abolished by bicuculline (10 µM, data not shown). The above data showed that GABAergic input to VB was dramatically reduced following RTN excision.

Comparison of propofol-mediated suppression in VB neurons in RTN-retained and RTN-removed slices

As shown above, removal of RTN markedly reduced spontaneous IPSC frequency, suggesting that there was a parallel decrease in GABA release. A decrease in receptor-bound GABA should attenuate the effects of GABA receptors, such as propofol. Therefore we compared the effect of propofol in RTN-removed and RTN-retained slices.

Single spike firing was initiated in a VB neuron in an RTN-retained slice (Fig. 9A, left), and bath application of propofol (1 µM) markedly decreased the number of spikes (right). In an RTN-removed slice, however, propofol suppression of spike firing was markedly attenuated (Fig. 9B). At a low concentration (0.6 µM), propofol had little effect in RTN-removed slices (Fig. 9C). Propofol decreased spike firing in a concentration-dependent manner. Concentration-effect curves were fit with the Hill function (Fig. 9D), yielding an estimated EC50 of 1.1 ± 0.3 (n = 40) and 3.5 ± 0.7 µM (n = 28) for RTN-retained and RTN-removed slices, respectively. There was a significant difference in the EC50 of RTN-retained and RTN-removed slices. The data clearly indicate that RTN is a critical structure for mediating propofol’s effects in thalamic circuits.

GABA receptors underlie the shunting effects of propofol in postsynaptic VB neurons

Since the GABAergic anesthetic pentobarbital can decrease input resistance via a non-GABAergic mechanism (Wan and Puil 2002), we examined mechanisms underlying the propofol-induced decrease in intrinsic excitability in VB neurons in RTN-retained slices. Input resistance in the absence of drug was 254 ± 8 MΩ (n = 90) in VB neurons. Bath application of propofol alone (1–10 µM) for 30 min suppressed spike firing (data not shown) and decreased input resistance in a concentration-dependent manner (Fig. 10A), although low concentrations (<3 µM) did not significantly affect input resistance. Normalized data are shown in Fig. 10A.

In another set of experiments, various compounds were tested to establish the mechanism for the propofol-induced decrease in input resistance. Propofol (6 µM) alone reduced
input resistance in VB neurons (n = 15). Preapplication of the GABA_A receptor antagonist bicuculline (10 μM, n = 15) did not significantly change input resistance, and subsequent co-application of propofol failed to affect input resistance (Fig. 10B). In a different group of neurons, preapplication of the GABA_B receptor antagonist 2-OH-salofen (100 μM, n = 10) had no effect, and addition of propofol decreased input resistance by nearly the same amount as propofol alone. Brief focal application (200 ms) of the GABA_A receptor agonist muscimol (1 μM in pipette) dramatically decreased input resistance and firing rate (traces not shown) in another 10 neurons, and this effect was completely reversed by addition of bicuculline. Finally, blockade of a persistent Na^+ conductance by TTX (1 μM) had no significant effect on input resistance in another 10 neurons, and addition of propofol (6 μM) significantly reduced input resistance during hyperpolarization or depolarization induced by current pulses (−60 and 60 pA). These data clearly indicated that the GABA_A receptor–mediated Cl^- conductance produced shunting inhibition in VB neurons during propofol application, and this mechanism is distinct from that of pentobarbital (Wan and Puil 2002).

DISCUSSION

This study showed that propofol was capable of enhancing intrinsic excitability, temporal summation, and spike firing in GABAergic RTN neurons. The enhanced GABAergic inhibitory input resulted in shunting inhibition in postsynaptic glutamatergic VB neurons. We also showed that thalamocortical suppression by propofol at clinically relevant concentrations critically depended on the presence and activation of the RTN. The observed contrasting responses to propofol in RTN and VB neurons seem consistent with the in vivo demonstration that propofol increases c-fos expression in a GABAergic nucleus and decreases its expression in a nonGABAergic nucleus in a sleep-promoting pathway (Nelson et al. 2002).

Propofol inhibition of SK channels enhanced RTN neuron excitability

Our study mainly focused on how propofol influenced intrinsic excitability in RTN neurons by testing changes in intrinsic responses (i.e., input resistance, temporal summation of subthreshold membrane voltage, and spike firing). In hippocampal neurons, increasing input resistance increases temporal summation at the soma (Magee 1998, 1999). Here, we showed that propofol markedly increased apparent input resistance (Figs. 1 and 2), which prolonged the decay time of intrinsic subthreshold voltage responses in RTN neurons (Table 1). Correspondingly, propofol increased temporal summation of subthreshold voltage responses. This effect was also mimicked and occluded by the specific SK channel blocker apamin.

The SK channel–mediated conductance is known to generate mAHF (Sah 1996; Stocker et al. 1999) and regulate neuronal excitability and tonic spike firing rate (Sah 1996; Stackman et
The SK2 channel protein is densely expressed in RTN but not in VB (Sailer et al. 2002; Stocker and Pedarzani 2000). Thus blockade of the SK channels during propofol application likely accounted for increased intrinsic excitability and spike firing rate in RTN neurons (Figs. 1–3), and this conclusion is consistent with previous findings that apamin-blockade of AHPs increases RTN neuron excitability (Bal and McCormick 1993; Debarbieux et al. 1998). The failure of propofol to affect fAHP in RTN is consistent with the insensitivity of the underlying potassium channel subtype to the low concentrations of propofol used here (Friederich and Urban 1999; Yamakura et al. 2001).

The SK channel–mediated conductance not only contributes to intrinsic excitability but also regulates synaptic efficacy during synaptic stimulation through the modulation of postsynaptic cells (Borde et al. 1999). We found that propofol markedly enhanced EPSP summation in response to corticothalamic pathway stimulation (Fig. 4, A and B). This enhancement was also primarily due to blockade of the SK channel in RTN neurons (Fig. 4C), but not due to a direct effect on glutamatergic axon terminals arising from corticothalamic neurons in RTN-retained and -removed slices (P < 0.05, Kolmogorov-Smirnov test). Data derived from B and C.

FIG. 8. Comparison of spontaneous IPSCs in VB neurons in RTN-retained and -removed slices. A: a photomicrograph of a horizontal live slice containing both VB and RTN (position marked by arrow). Solid line indicates the cut through the slice to remove RTN and other structures. B: spontaneous IPSCs (sIPSCs) recorded from an RTN-retained slice (top), and group data (bottom) showing the sIPSC frequency in such slices (n = 30). Error bars are omitted for clarity. C: sIPSCs recorded from an RTN-removed slice (top; same scale as in B); group data (bottom) showing the sIPSC frequency in RTN-removed slices (n = 12). Note that the sIPSC frequency was dramatically reduced in RTN-removed slices. D: cumulative amplitude distributions from pooled results for sIPSCs in RTN-retained and RTN-removed slices. Difference in cumulative probability is significant between RTN-retained and -removed slices (P < 0.05, Kolmogorov-Smirnov test). Data derived from B and C.

FIG. 9. Comparison of propofol’s effects in VB neurons in RTN-retained and -removed slices. A: left: sample trace showing tonic spike firing recorded from a VB neuron in an RTN-retained slice. Spikes are truncated for clarity in all panels. Right: bath application of propofol decreased spike firing. B: left: spike firing recorded from another VB neuron in an RTN-removed slice, and (right) inhibitory effects of propofol were markedly attenuated. C: group data showing that a significant inhibitory effect produced by a low concentration (0.6 μM) of propofol was observed only in RTN-retained slices, but not in RTN-removed slices. *P < 0.05, 1-way ANOVA vs. control, n = 12/each. D: concentration-effect curves for inhibitory effects of propofol on VB neuron firing in RTN-retained and -removed slices. There is a significant difference in effects of propofol between RTN-retained and -removed groups, P < 0.05, t-test, n = 12–15/each data point.
mediated by GABA<sub>A</sub> receptor chloride channels, rather than other ion channels.

...GABAergic terminals, if any, is not a major mechanism underlying GABA release during propofol application. Since propofol (0.6 μM) significantly increased both GABAergic synaptic transmission at RTN-VB synapses (Fig. 5A) and the frequency of large IPSCs (Fig. 5C), we conclude that low concentration propofol increases the spontaneous IPSC frequency (or GABA release) by increasing RTN neuron spike firing rate (Figs. 1 and 2). This conclusion is consistent with other observations showing that low concentrations of propofol have little effect on the processes governing transmitter release from axon terminals (Mantz et al. 1995; Olcese et al. 1994; Shirasaka et al. 2004; Takei et al. 2003; Westphalen and Hemmings 2003; Ya Deau et al. 2003; reviewed by Richards 2002).

**RTN input is important for mediating the effects of propofol in thalamic circuits**

We also showed that propofol suppression of postsynaptic VB neurons depends on GABAergic input. Given that action potential–dependent GABAergic inhibition in VB neurons, removal of the RTN should attenuate such inhibition. Indeed, removing the RTN completely abolished the suppression elicited at low concentrations (<1 μM) and decreased the overall potency of propofol by 218% (i.e., increasing EC<sub>50</sub> from 1.1 to 3.5 μM). Therefore the presence of the RTN is critical for propofol to elicit thalamocortical suppression at clinically relevant concentrations. As concentrations increased, propofol continued to suppress VB neurons in RTN-removed slices (Fig. 9D). This is likely due to residual GABA release from GABAergic terminals remaining in VB and possibly direct activation of GABA<sub>A</sub> receptors by propofol (Sanna et al. 1995). Several sets of intrinsic conductances (e.g., I<sub>H</sub>, I<sub>g</sub>) are known to regulate VB neuron firing (Steriade et al. 1997), and the effect of propofol on these conductances warrants further study.

**Postsynaptic effects in VB neurons are mediated by GABA<sub>A</sub> receptors**

As shown in Figs. 7 and 9, propofol suppressed tonic spike firing in VB neurons. This suppression was associated with a decrease in apparent input resistance in VB neurons (Fig. 10A). The shunting inhibition, mimicked by the GABA<sub>A</sub> receptor agonist muscimol, could be blocked by the antagonism of GABA<sub>A</sub>, but not GABA<sub>B</sub>, receptors. These data indicate that the postsynaptic shunting inhibition in glutamatergic VB neurons resulted from a propofol-mediated increase in the GABA<sub>A</sub> receptor–mediated chloride conductance and is comparable with the effect seen in hippocampal neurons (Orser et al. 1994). In summary, propofol activation of RTN neurons via blockade of SK channels represents a novel presynaptic mechanism of action. As a result of RTN activation, GABAergic transmission increased at RTN-VB synapses, and this increased the postsynaptic GABA<sub>A</sub> conductance in VB neurons, thereby “gating” thalamocortical spike output (Le Masson et al. 2002). Our results support the hypothesis that thalamocortical circuits are an important target for the general anesthetic propofol (Bonhomme et al. 2001; Fiset et al. 1999; Hofbauer et al. 2004; Menon 2001).
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REFERENCES


Antkowiak B. Different actions of general anesthetics on the firing patterns of neocortical neurons mediated by the GABA_A receptor. Anesthesiology 91: 500–511, 1999.


Borde M, Bonansco C, and Buno W. The activity-dependent potentiation of the slow Ca^{2+}-activated K^{+} current regulates synaptic efficacy in rat CA1 pyramidal neurons. Pfluegers Arch 437: 261–266, 1999.


Franks NP and Lieb WR. Which molecular targets are most relevant to general anaesthesia? Toxicol Lett 100–101: 1–8, 1998.


Liu XB, Bolea S, Golshan P, and Jones EG. Differentiation of corticothalamic and collateral thalamocortical synapses on mouse reticular nucleus neurons.


Shirasaka T, Yoshimura Y, Qiu DL, and Takasaki M. 

Sherman SM and Guillery RW. 

Semyanov A and Kullmann DM. 

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Liu XB, Warren RA, and Jones EG. 

Nishikawa K and MacIver MB. 

Ohara PT and Lieberman AR. 

The *AJ*, Hadingham KL, Hutson PH, Belelli D, Lambert JJ, Dawson GR, 


