

Propofol Facilitates Glutamatergic Transmission to Neurons of the Ventrolateral Preoptic Nucleus

Ke Y. Li, M.D., Ph.D., Yan-zhong Guan, M.D., Ph.D.,* Kresimir Krnjević, M.D., Ph.D.,† Jiang H. Ye, M.D., M.Sc.‡*

Background: **There is much evidence that the sedative component of anesthesia is mediated by γ-aminobutyric acid type A ($GABA_A$) receptors on hypothalamic neurons responsible for arousal, notably in the tuberomammillary nucleus. These $GABA_A$ receptors are targeted by γ-aminobutyric acid–mediated (GABAergic) neurons in the ventrolateral preoptic area (VLPO): When these neurons become active, they inhibit the arousal-producing nuclei and induce sleep. According to recent studies, propofol induces sedation by enhancing VLPO-induced synaptic inhibition, making the target cells more responsive to $GABA_A$. The authors explored the possibility that propofol also promotes sedation less directly by facilitating excitatory inputs to the VLPO GABAergic neurons.**

Methods: **Spontaneous excitatory postsynaptic currents were recorded from VLPO cells—principally mechanically isolated, but also in slices from rats.**

Results: **In isolated VLPO GABAergic neurons, propofol increased the frequency of glutamatergic spontaneous excitatory postsynaptic currents without affecting their mean amplitude. The action of propofol was mimicked by muscimol and prevented by gabazine, respectively a specific agonist and antagonist at $GABA_A$ receptors. It was also suppressed by bumetanide, a blocker of Na^+–K^+–Cl^- cotransporter-mediated inward Cl^- transport. In slices, propofol also increased the frequency of spontaneous excitatory postsynaptic currents and, at low doses, accelerated firing of VLPO cells.**

Conclusion: **Propofol induces sedation, at least in part, by increasing firing of GABAergic neurons in the VLPO, indirectly by activation of $GABA_A$ receptors on glutamatergic afferents: Because these axons/terminals have a relatively high internal Cl^- concentration, they are depolarized by GABAergic agents such as propofol, which thus enhance glutamate release.**

THE cellular and molecular mechanisms underlying the effects of general anesthetics are not well understood. The sedative component of anesthesia seems to be mediated by γ-aminobutyric acid type A receptors ($GABA_A$Rs) in an endogenous sleep pathway,[1,2] the relevant γ-aminobutyric acid–mediated (GABAergic) neurons being located in the ventrolateral preoptic area (VLPO).[3–8] Recently, Zecharia *et al.*[2] showed that GABAergic agents such as muscimol and propofol potentiate the GABAergic inhibition of orexinergic perifornical[9] and histaminergic tuberomammillary neurons.[6] Together with other monoaminergic and cholinergic projections to the cortex, the neurons in tuberomammillary nuclei directly promote cortical arousal,[10,11] and all are activated by orexinergic neurons.[12] Therefore, their inhibition by VLPO neurons is pivotal in the initiation of sleep.[13,14] Although the activity of VLPO neurons is strongly influenced by circadian fluctuations in input from suprachiasmatic cells,[15] the detailed mechanisms underlying activation of VLPO neurons are largely unexplored.[16]

General anesthetics are known to have marked effects on synaptic transmission. Most previous studies found that propofol enhances the function of inhibitory $GABA_A$Rs[17–20] and may depress the release of glutamate, the major excitatory neurotransmitter.[21] However, *in vitro* electrophysiologic studies have shown that, in several brain areas, activation of presynaptic $GABA_A$Rs have a depolarizing effect that enhances glutamatergic transmission.[22–25] We hypothesized that by potentiating the depolarizing action of presynaptic $GABA_A$Rs, propofol may increase glutamatergic transmission in the VLPO and thus increase the activity of VLPO GABAergic neurons. To test this hypothesis, we recorded spontaneous excitatory postsynaptic currents (sEPSCs) in rat VLPO GABAergic neurons, either in slices or isolated by a mechanical, enzyme-free procedure. These neurons preserve some functional glutamate-releasing terminals after isolation.

Materials and Methods

All experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and they were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey, Newark, New Jersey. The experiments were performed on brains from Sprague-Dawley rats.

Slice Preparation and Mechanical Dissociation

The hypothalamic slices were prepared as described previously.[26,27] Briefly, Sprague-Dawley rats (aged 10–28 postnatal days) were anesthetized and then decapitated. Coronal slices (300 μm thick) were cut using a VF-200 slicer (Precisionary Instruments Inc., Greenville, NC). They were prepared in ice-cold glycerol-based artificial cerebrospinal fluid containing 250 mM glycerol, 1.6 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose and saturated with 95% O_2–5% CO_2 (carbogen).[28] Slices were allowed to recover for at least 1 h in a holding chamber at 32°C in carbogen-saturated regular

* Postdoctoral Fellow, ‡ Professor, Departments of Anesthesiology and Pharmacology and Physiology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School. † Professor, Department of Physiology, McGill University, Montreal, Quebec, Canada.

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Address correspondence and reprint requests to Dr. Ye: Department of Anesthesiology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, 185 South Orange Avenue, Newark, New Jersey 07103. ye@umdnj.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue.

artificial cerebrospinal fluid, which has the same composition as glycerol-based artificial cerebrospinal fluid, except that glycerol was replaced by 125 mM NaCl.

Neurons with functional presynaptic terminals attached were obtained by mechanical dissociation (fig. 1A), as previously described,[29] with some modifications.[28,30] A slice containing the VLPO was transferred to a 35-mm culture dish (Falcon, Rutherford, NJ) and held down with a flat U-shaped wire. The dish was filled with standard external solution containing 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose (320 mOsm, pH adjusted to 7.3 with Tris base). Under an inverted microscope (Nikon, Tokyo, Japan), we identified VLPO by its stereotaxic coordinates,[31] and located the VLPO cells that project to the noradrenergic locus ceruleus and the histaminergic tuberomammillary nucleus.[3,6,32] A fire-polished pipette, held by a micromanipulator, lightly touched the surface of the VLPO region and vibrated horizontally at 15–20 Hz for 2–5 min. After 20 min, isolated neurons that adhered to the bottom of the dish were kept for electrophysiologic recordings at room temperature (21°–22°C). These mechanically dissociated neurons often preserve functional nerve terminals, including some that release glutamate.[29,30]

Electrophysiologic Recordings

Electrical signals were obtained in whole cell configurations with an Axon 200B amplifier or a MultiClamp 700A (Molecular Devices Co., Union City, CA), a Digidata 1320A A/D converter (Molecular Devices Co.), and pCLAMP 9.2 software (Molecular Devices Co.). Data were filtered at 1 kHz and sampled at 5 kHz. The patch electrodes had a resistance of 2–5 MΩ when filled with the pipette solution, containing 135 mM CsF, 5 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, 2 mM Mg adenosine-5′-triphosphate, and 0.2 mM guanosine-5′-triphosphate (for voltage clamp). For current clamp recordings, the CsF in the above pipette solution was replaced by K-gluconate. The pH of the pipette solution was adjusted to 7.2 with Tris base. Neurons were voltage clamped at −60 mV to record α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor–mediated spontaneous and miniature excitatory postsynaptic currents (sEPSCs and mEPSCs).

In several experiments, a single coronal slice was transferred into a 0.4-ml recording chamber where it was held down by a platinum ring. Warm carbogenated artificial cerebrospinal fluid flowed through the bath (1.5–2.0 ml/min). Under infrared video microscopy (E600FN; Nikon), we recorded from neurons located in the core of VLPO, where a cluster of sleep-active neurons were originally identified, as illustrated in figure 1.[5,6] Cells were deemed of the sleep promoting type according to previously established criteria[3]: First, they were triangular in shape (fig. 1A1), in contrast to the fusiform, non–low-threshold spike type (fig. 1A2); second, when recorded in current clamp mode, they generated characteristic low-threshold spikes

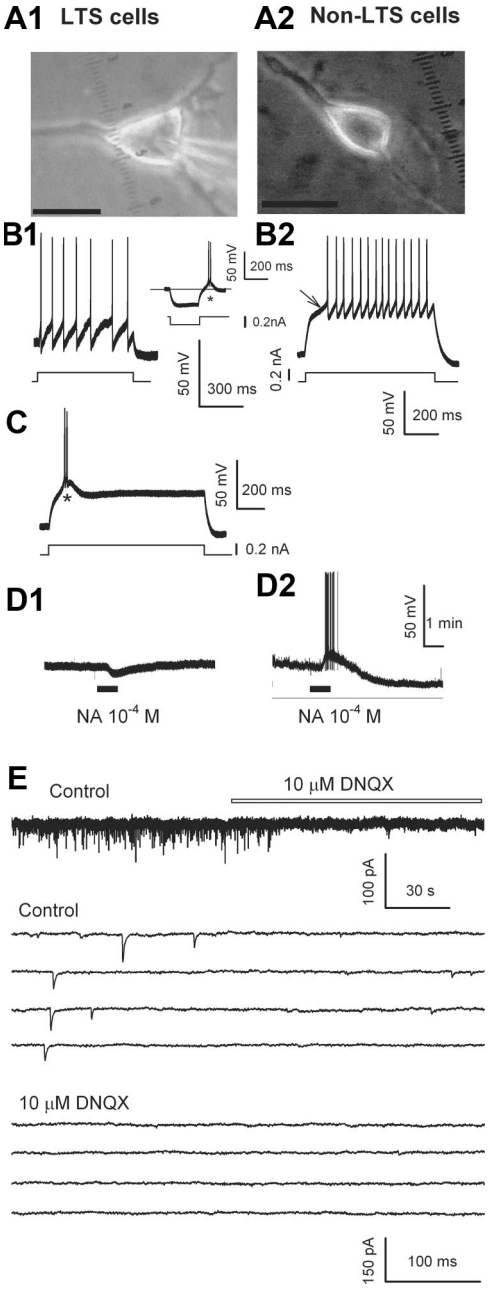

Fig. 1. Mechanically dissociated two types of ventrolateral preoptic nucleus neurons and glutamatergic excitatory postsynaptic currents. (*A*) Typical mechanically dissociated ventrolateral preoptic nucleus neuron: Note triangular shape characteristic of γ-aminobutyric acid–mediated cells with low-threshold spikes (LTS)[3] (*A1*, with patch pipette in place), *scale bar*: 20 μm, and bipolar fusiform non-LTS neuron (*A2*), *scale bar*: 15 μm. LTSs occurred when the multipolar cell was depolarized from a hyperpolarizing level (at * in *inset* in *B1*, and in trace *C*). This same neuron was hyperpolarized by 100 μM noradrenaline (NA; *D1*). (*B2*) A slow voltage response toward the first action potential (*arrow*) recorded from a bipolar, fusiform non-LTS neuron (*A2*). This neuron was depolarized, and firing was induced by 100 μM NA (*D2*). (*E*) Spontaneous excitatory postsynaptic currents recorded in a multipolar neuron were completely blocked by 2,3-dihydroxy-6,7-dinitroquinoxaline (DNQX, 10 μM), indicating that these events were mediated by AMPA/kainate–type glutamate receptors. Some traces are shown on an expanded time scale. For all figures, *horizontal bars* indicate period of drug applications.

in response to a depolarizing pulse or after a hyperpolarizing current step (figs. 1B1 and C; fig. 1B2 shows the high-threshold spikes of non–low-threshold spike cells); and finally, they were inhibited by noradrenaline (fig. 1D1). Triangular-shaped cells inhibited by noradrenaline always displayed low-threshold spikes. By contrast, noradrenaline excited a non–low-threshold spike neuron (fig. 1D2). The series resistance (15–30 MΩ) or input resistance (300–500 MΩ) was monitored throughout the whole cell recording, and data were discarded if the resistance changed by more than 20%. All these recordings were made at 32°C, maintained by an automatic temperature controller (Warner Instruments, Hamden, CT).

Chemicals and Applications

The chemicals, including 2, 6-diisopropylphenol (propofol), gabazine (SR 95531), 2,3-dihydroxy-6,7-dinitroquinoxaline (DNQX), tetrodotoxin, bicuculline, and bumetanide were obtained from Sigma-Aldrich Chemical Company (St Louis, MO). Drugs were added to the superfusate or applied to a cell by a fast perfusion system (Y tube). Solutions in the vicinity of a neuron could be completely exchanged within 40 ms without damaging the seal.[33]

Statistical Analysis

sEPSCs and mEPSCs were counted and analyzed with Clampfit 9.2 (Molecular Devices Co.); sEPSCs and mEPSCs were screened automatically (6-pA amplitude threshold), checked visually, and accepted or rejected according to their rise and decay times. The frequency and amplitude of all events, during and after drug application, were compared with the mean of the values observed during the initial control period. Cumulative probability plots of the incidence of various interevent intervals and amplitudes, recorded under different conditions from the same neuron, were subjected to the Kolmogorov–Smirnov test. For other plots, data obtained over 1-min periods at the peak of a drug response were compared with the average values of frequency (or amplitude) of the sEPSC (or mEPSC) recorded during the initial control period (1–4 min). Data are expressed as mean (±SEM). sEPSCs were fitted with a standard single exponential equation (Clampfit 9.2) to determine the time constant of decay from the EPSC peak. The statistical significance of drug effects was assessed by a paired two-tailed *t* test on normalized data with Sigma Plot (Systat Software Inc., San Jose, CA). Values of $P < 0.05$ were considered significant.

Results

Spontaneous EPSCs Recorded in Neurons Mechanically Dissociated from VLPO

In VLPO, two thirds of the neurons have a characteristic triangular and multipolar shape and low-threshold-spikes; moreover, because these cells contain the GABA-synthesizing enzyme glutamic acid decarboxylase and are inhibited by noradrenaline and carbachol, they correspond to the GABAergic VLPO cells known to be active during sleep.[3,8] In the current study, we recorded from such multipolar, triangular-shaped neurons within VLPO (fig. 1). Most of the experiments were performed on mechanically dissociated neurons (fig. 1A1), which combine several advantages: good space clamp; preservation of functioning synaptic terminals, including some that release glutamate; and better control of the surrounding solution.[29,30,34,35] The traces in figures 1B–D were recorded under current clamp. The voltage traces in figures 1B1, C, and D were from a triangular-shaped, multipolar VLPO neuron. Depolarization from a relatively hyperpolarized level was followed by the characteristic depolarizing hump and burst of firing (inset in figs. 1B1 and C), and noradrenaline-induced membrane hyperpolarization (fig. 1D1). Conversely, in the bipolar, fusiform VLPO neuron (fig. 1A2), in response to a depolarization pulse there was a slow voltage rise toward the first action potential (fig. 1B2, arrow) and noradrenaline-induced membrane depolarization and firing (fig. 1D2). Under voltage clamp, sEPSCs were recorded with the CsF-based internal solution at a holding potential of −60 mV. Under these conditions, postsynaptic responses to GABA or glycine are suppressed,[36] allowing us to monitor changes in frequency and amplitude of the isolated EPSCs.[30,37,38] DNQX (10 μM), an antagonist of AMPA/kainate receptor, reversibly blocked the spontaneous synaptic events (fig. 1E), confirming that these VLPO neurons receive glutamatergic excitatory inputs.[15]

Propofol Increases sEPSC Frequency in Neurons Mechanically Dissociated from VLPO

Propofol (1–100 μM) was applied to isolated VLPO neurons by a fast perfusion system (Y-tube).[39] Propofol (10 μM) did not significantly change the holding current, whereas it robustly but reversibly increased the frequency of sEPSCs (fig. 2). A second application of propofol had a comparable effect (88 ± 7% of first response; n = 8, $P > 0.5$; figs. 2A and B). As depicted by averaged traces recorded before and during propofol application (fig. 2C) and the dose–response data in figure 2E, propofol (10 μM) did not significantly alter the mean amplitude of sEPSCs (7.6 ± 1.2 pA in control and 10.3 ± 1.8 pA in presence of propofol; $P > 0.05$, n = 30 events). The superimposed normalized mean traces at right in figure 2C illustrate the absence of any change in sEPSC kinetics: The decay time constant of sEPSCs was not significantly altered (control: 3.9 ± 1.3 ms, propofol: 2.3 ± 0.4 ms; $P = 0.13$, by *t* test). The effect of propofol on sEPSC frequency depended on its concentration, as shown in figure 2D. The apparent EC_{50} for propofol was 4.2 μM. These results are consistent with a presynaptic mecha-

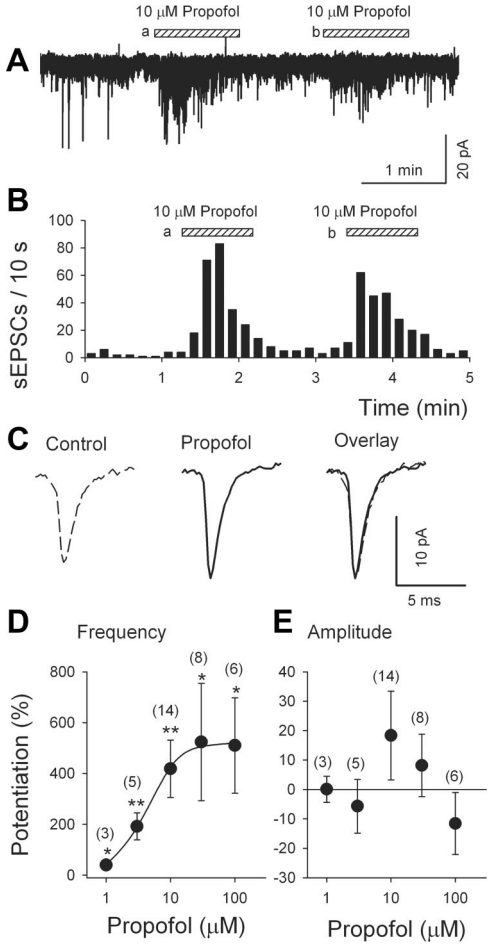

Fig. 2. Propofol increases the frequency (but not amplitude) of spontaneous excitatory postsynaptic currents (sEPSCs) recorded in mechanically dissociated multipolar ventrolateral preoptic nucleus neurons. (*A*) Increased incidence of sEPSCs during two applications of 10 μM propofol. (*B*) Time course of propofol-induced enhancement of sEPSC frequency (same data as illustrated in *A*). (*C*) Averaged traces show only minor change in amplitude, which was not significant (see *E*). Overlay of normalized traces indicates no change in sEPSC kinetics in the presence of propofol (10 μM) (n = 30 events). Dose–response relation for propofol-induced changes in sEPSC frequency (*D*) and amplitude (*E*). *Solid line* in *D* is least square fit of the frequency data to the Michaelis–Menten equation: $P = (P_{MAX} \times C^n)/(C^n + EC_{50}^n)$, where P, P_{MAX}, C, EC_{50}, and n are percent potentiation, maximal potentiation, propofol concentration, concentration of propofol at which the potentiation of sEPSC frequency is 50% of maximum, and the Hill coefficient, respectively. The EC_{50} was 4.2 μM. Each *circle* represents the mean ± SEM of results from 3 to 14 cells. * $P < 0.05$, ** $P < 0.01$ by paired *t* test for propofol *versus* prepropofol.

nism of propofol action, enhancing glutamatergic transmission by increasing glutamate release.

Propofol Acts Only on sEPSCs That Are Suppressed by Tetrodotoxin and Cd^{2+}

We next examined how block of synaptic transmission affects the propofol-induced facilitation of sEPSC frequency. First, we examined the effect of 1 μM tetrodotoxin, a blocker of voltage-dependent Na^+ channels. Tetrodotoxin (1 μM) sharply decreased sEPSC frequency to 38 ± 10% of control (from 3.4 ± 1.2 Hz in control to 1.1 ± 0.6 Hz in tetrodotoxin; n = 5, $P < 0.01$), but a moderate reduction in sEPSC amplitude was not significant (to 75 ± 7% of control, from 17 ± 5 pA to 13 ± 4 pA; n = 5, $P > 0.05$). In the presence of 1 μM tetrodotoxin, propofol (10 μM) did not significantly alter either the frequency or the amplitude of the remaining EPSCs (presumably mEPSCs) (figs. 3A–C). These results indicate that propofol acts only on sEPSCs generated by ongoing activity of tetrodotoxin-sensitive Na^+ channels.

In further experiments, we applied Cd^{2+}, a general blocker of voltage-dependent Ca^{2+} channels, which also suppresses synaptic transmission. Indeed, acting somewhat like tetrodotoxin, 100 μM Cd^{2+} reduced sEPSC frequency by 36 ± 10% ($P = 0.03$, n = 5) and amplitude by 32 ± 9% ($P = 0.02$, n = 5), and in its presence, 10 μM propofol did not significantly alter the frequency of the remaining EPSCs (figs. 3D–F). Judging by these results, propofol targets only EPSCs generated by Na^+ channel–dependent terminal depolarization and the resulting Ca^{2+} influx.

The Propofol-induced Increase in Glutamatergic Activity Is Eliminated by a $GABA_AR$ Antagonist and Mimicked by a $GABA_AR$ Agonist

It is well known that propofol enhances the function of $GABA_A$Rs, in particular those containing β_2 and β_3 subunits,[40] and that activation of presynaptic $GABA_A$Rs enhances glutamate release in several brain areas.[22–25] To test for possible involvement of $GABA_A$Rs in the acceleration of sEPSC frequency, we applied gabazine (10 μM), a specific antagonist of $GABA_A$Rs. As shown in figures 4A–C, in the presence of gabazine, 10 μM propofol caused a mild reduction in sEPSC frequency (−17.9 ± 2.1%, n = 6), presumably mediated by its $GABA_A$R-independent depressant effect on glutamate release.[21] To further confirm the role of $GABA_A$Rs in the facilitation of sEPSCs, we applied muscimol, the specific $GABA_A$R agonist. In five cells tested, 10 μM muscimol significantly increased sEPSC frequency, but not amplitude (figs. 4C–E).

The Propofol-induced Increase in Glutamatergic Activity Is Attenuated by an Antagonist of Na^+–K^+–$2Cl^-$ Cotransporters

We have shown that voltage-dependent Na^+ and Ca^{2+} channels, as well as $GABA_A$Rs, are involved in the propofol-induced facilitation of sEPSC frequency. These data suggest that the activation of presynaptic $GABA_A$Rs depolarizes some glutamatergic nerve terminals by generating Cl^- efflux. This implies that the intraterminal Cl^- concentration ($[Cl^-]$) is relatively high, perhaps owing to inwardly directed Cl^- transport systems *via* Na^+–K^+–$2Cl^-$ cotransporters (NKCCs) and/or the Cl^-–HCO_3^- exchanger.[41–43] In peripheral sensory axons and immature or injured CNS neurons, NKCC increases intracellular $[Cl^-]$; hence, in these cells, GABA induces depolarization.[43] Indeed, in some other hypothalamic

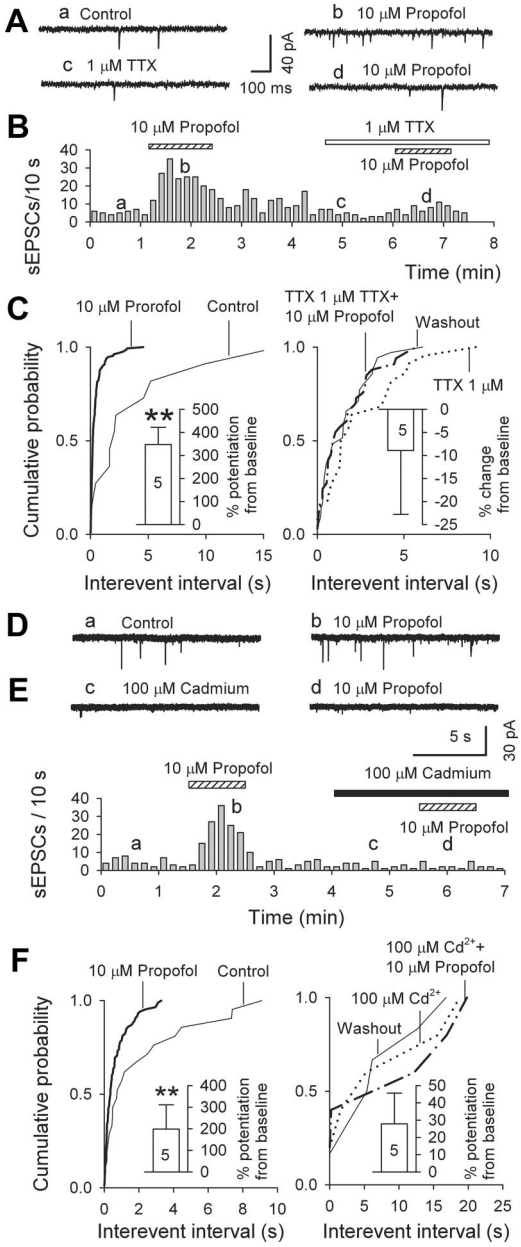

Fig. 3. Miniature excitatory postsynaptic currents, in mechanically isolated ventrolateral preoptic nucleus neurons recorded in the presence of tetrodotoxin (TTX) or Cd^{2+}, are not sensitive to propofol. (*A–C*) Effects of propofol on spontaneous excitatory postsynaptic currents (sEPSCs) recorded in the absence and presence of 1 μM tetrodotoxin. (*A*) Original traces of spontaneous EPSCs obtained at times indicated by *lowercase letters* in *B*. (*B*) Time course of changes in sEPSC frequency. (*C*) Cumulative probability plots of intervals between sEPSCs show that the sharp effect of propofol disappears in the presence of tetrodotoxin (n = 5). *Insets* are mean changes (± SEM): $^{**}P < 0.01$ by paired *t* test for propofol *versus* prepropofol. (*D–F*) Effects of propofol on sEPSCs recorded in the absence and presence of 100 μM Cd^{2+}. (*D*) Original traces of sEPSCs. (*E*) Time course of changes in sEPSC frequency. (*F*) Cumulative probability plots of intervals between sEPSCs: As indicated by *inset histograms* (mean ± SEM, n = 5), the effect of propofol was no longer significant in the presence of 100 μM Cd^{2+}. $^{**}P < 0.01$ by paired *t* test for propofol *versus* prepropofol values.

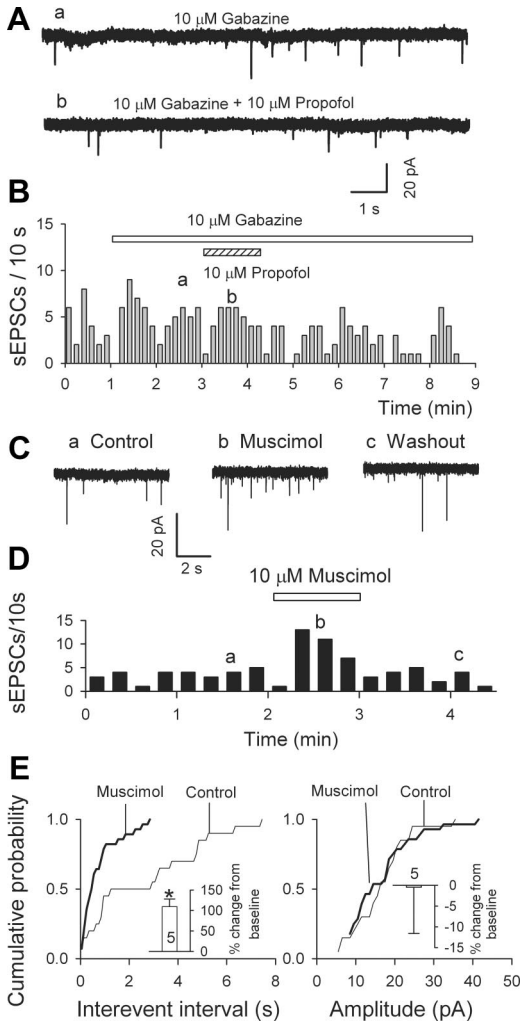

Fig. 4. The action of propofol is mediated *via* γ-aminobutyric acid type A receptors: Propofol-induced increase in the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) was abolished by the selective γ-aminobutyric acid type A receptor antagonist gabazine and mimicked by the selective γ-aminobutyric acid type A receptor agonist muscimol. (*A–C*) Gabazine effects. (*A*) Traces from a neuron isolated from ventrolateral preoptic nucleus show that propofol (10 μM) had no effect on sEPSCs in the presence of gabazine (10 μM). (*B*) Time course of above data. (*C–E*) Like propofol, muscimol increases sEPSC frequency but not amplitude. (*C*) Traces recorded before (*a*, control), during (*b*, 10 μM muscimol), and after washout (*c*). (*D*) Time course of effect of muscimol on one cell. (*E*) *Cumulative plots* and *insets* (mean ± SEM from five cells) show increase in sEPSC frequency (*left panel*) but not amplitude (*right panel*). $^{*}P < 0.05$ by paired *t* test for muscimol *versus* control (n = 5).

neurons, NKCC is responsible for a relatively high $[Cl^-]$ in glutamatergic nerve terminals.[44,45] To determine whether a high internal $[Cl^-]$, maintained by NKCC, is essential for the propofol-induced facilitation of sEPSC frequency, we tested propofol in the presence of 10 μM bumetanide, which selectively blocks NKCC[43,46] (but not GABA receptors[47]). As illustrated in figure 5 by original traces in A, the plot in B, and mean data in C, after 5 min of 10 μM bumetanide application, 10 μM propofol did not significantly alter the frequency of

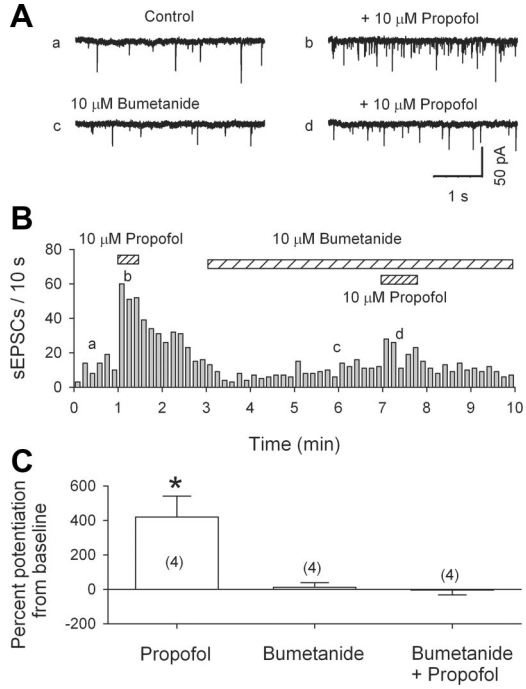

Fig. 5. Suppression of outward Cl^- gradient eliminates excitatory action of propofol. (*A*) Traces from a dissociated ventrolateral preoptic nucleus neuron show a much smaller effect of propofol (10 μM) on the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in the presence of 10 μM bumetanide, a blocker of Na^+–K^+–Cl^- cotransporter. (*B*) Time course of changes in frequency. (*C*) Means ± SEM from four cells. * $P < 0.05$ by paired *t* test for propofol *versus* prepropofol data.

sEPSCs. The effect of propofol slowly recovered to control values after washing out bumetanide (data not shown). This result suggests that functional NKCCs are present on the juxtaterminal portion of glutamatergic axons, where they maintain a relatively high internal $[Cl^-]$.

Propofol Raises the Frequency Both of sEPSCs and of Ongoing Firing of VLPO Neurons in Brain Slices

We confirmed that propofol modulates glutamate release onto VLPO neurons under more physiologic conditions in slices (fig. 6). Propofol was tested on multipolar VLPO neurons, identified by the standard criteria (fig. 6A). As illustrated by the traces (fig. 6B), plot (fig. 6C), and histograms (fig. 6D), 2 μM propofol robustly increased the frequency (but not amplitude) of glutamatergic sEPSCs in these VLPO neurons. Having established that propofol (2 μM) facilitates glutamatergic transmission in slices, we next assessed the physiologic consequences of the action of propofol by examining the ongoing discharge of VLPO neurons. As shown in figures 6E and F, 0.1 μM propofol significantly increased the frequency of spontaneous firing of VLPO neurons in brain slices. Interestingly, as illustrated in figures 6G and H, at a higher concentration (2 μM) propofol had a sharp depolarizing action, which inactivated firing.

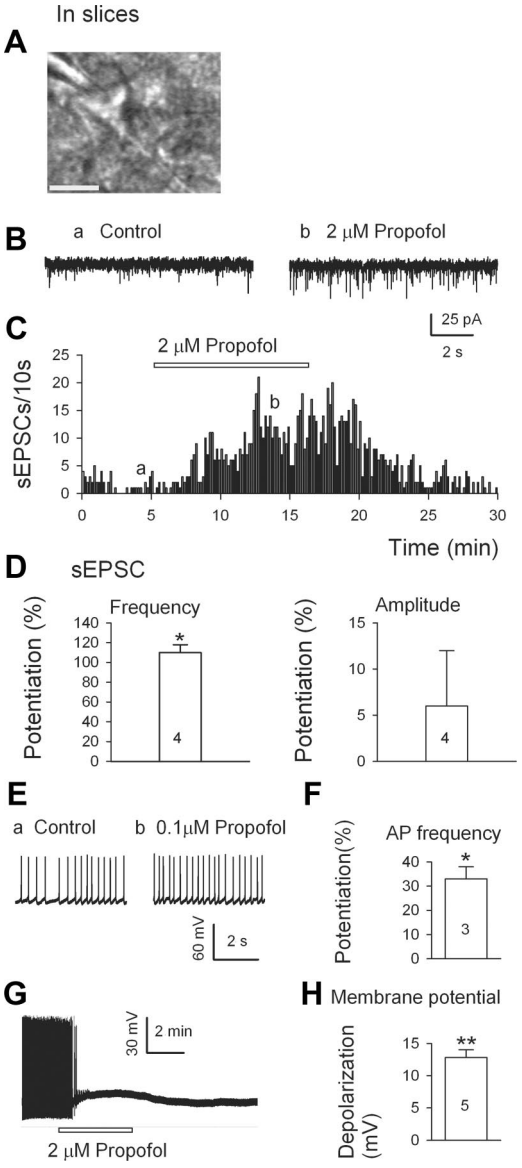

Fig. 6. Propofol increases the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and firings in multipolar neurons in ventrolateral preoptic nucleus in slices. (*A*) A triangular-shaped neuron in ventrolateral preoptic nucleus with patch pipette in place. *Scale bar*: 20 μm. (*B*) Propofol, 2 μM, significantly increased the frequency of sEPSCs in such a multipolar neuron in ventrolateral preoptic nucleus. (*C*) Time course of propofol-induced enhancement of sEPSC frequency. (*D*) Mean effects (±SEM, n = 4 cells) of 2 μM propofol on frequency and amplitude of sEPSCs. (*E*) Typical current traces and mean effects (±SEM, n = 3 cells) (*F*) of spontaneous action potential (AP) frequency of neurons in ventrolateral preoptic nucleus show that 0.1 μM propofol significantly increases the firing rate. (*G*) Typical current traces and mean effects (±SEM, n = 5 cells) (*H*) of neurons in ventrolateral preoptic nucleus show that 2 μM propofol significantly depolarizes the neuron. * $P < 0.05$, ** $P < 0.01$, respectively, by paired *t* test for propofol *versus* prepropofol data.

Discussion

The central finding of this study is that propofol—at concentrations (1–10 μM) similar to those found effective in potentiating $GABA_A$Rs in previous studies

(*e.g.*,[48–51])—raises the frequency of spontaneous glutamatergic transmission to VLPO neurons, both isolated and in slices. This is the first electrophysiologic demonstration of the potentiating effect of propofol on glutamatergic transmission at the single cell level. An interesting parallel observation is that propofol, over a range of moderate concentrations (10–13 μM), selectively increases Ca-dependent glutamate release from cortical synaptosomes.[52] The VLPO neurons under study were multipolar in shape, generated low-threshold spikes, and were inhibited by noradrenaline, and therefore belonged to the GABAergic population[3] that projects to and inhibits the principal deep nuclei responsible for cortical and behavioral arousal.[8] According to previous studies,[2,20] the sedative action of propofol is mediated especially by enhanced GABAergic inhibition of the cells of the tuberomammillary nuclei. Specifically, Zecharia *et al.*[2] recently found that propofol enhances GABAergic inhibition of these cells by increasing the duration of inhibitory synaptic responses. This did not exclude the possibility that propofol also increases the excitability of VLPO neurons in some indirect manner, *e.g.*, by disinhibition.[1] The current findings show that propofol increases ongoing excitation of the GABAergic VLPO neurons by enhancing glutamate release. Clearly, this mechanism could be a significant component of the sedative action of propofol.

Our recordings of AMPA receptor–mediated glutamatergic sEPSCs in the VLPO neurons are in keeping with a previous report.[15] A presynaptic target for the action of propofol was indicated by the increase in sEPSC frequency and insignificant changes in the mean amplitude of sEPSCs and mEPSCs. The presence of $GABA_A$Rs on or near the glutamatergic terminals was supported by the demonstration that the effect of propofol was mimicked by muscimol, a $GABA_A$R agonist, and blocked by gabazine, a $GABA_A$R antagonist. The fact that activation of these $GABA_A$Rs enhances glutamate release is readily explained if the Cl^- concentration in these glutamatergic axons/terminals is relatively high; in which case, opening the presynaptic Cl^- channels would induce Cl^- efflux and thus membrane depolarization. The higher internal Cl^- concentration seems to be the result of the activity of axonal NKCC-mediated inward Cl^- transport, because bumetanide—a selective inhibitor of Cl^- transport that does not block $GABA_A$ receptors[47]—prevented the action of propofol. On the other hand, in view of the block of propofol's effect by tetrodotoxin, the depolarization directly caused by presynaptic $GABA_A$R–induced Cl^- efflux was unable to induce glutamate release, either because it was of insufficient magnitude to activate Ca^{2+} influx into the terminal or because the operative $GABA_A$Rs are situated at some distance, requiring the conduction of tetrodotoxin-sensitive action potentials. In either case, the participation of voltage-dependent Na^+ channels was crucial.

Previous studies on spinal cord, hippocampus, thalamus, and neocortex have not reported an excitatory effect of propofol on glutamatergic EPSCs. The simplest explanation could be that in contrast to previous authors, we recorded EPSCs in the absence of $GABA_A$R antagonists, which eliminate this propofol action (as shown by our observations in the presence of gabazine).

Previous studies suggest that some $GABA_A$R agonists promote sleep by inhibiting the histaminergic cells in the tuberomammillary nucleus and weakly activating the VLPO *via* agonist binding to the α_1 subunit of $GABA_A$Rs, whereas gaboxadol (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol [THIP]) binds to the $\alpha_4\delta$ subunits, potentially promoting sleep by activation of the VLPO.[16] However, how activation of $GABA_A$Rs could excite VLPO neurons was not determined. Our study may provide an answer to this question. Future research will define the relevant subunits of $GABA_A$Rs and whether the $\alpha_4\delta$ subunits in particular mediate the propofol-induced potentiation of sEPSCs of VLPO neurons.

In the current study, we tested 2 μM propofol on both mechanically dissociated neurons and slices. Its effects on sEPSCs (in whole cell voltage clamp recordings) were similar in both preparations, but the facilitation of sEPSCs occurred more quickly in mechanically dissociated neurons than in slices, as might be expected in view of the slow diffusion of propofol in slices.[53] On the other hand, in current clamp recordings of ongoing firing in slices, we found that even 0.1 μM propofol significantly increased the firing rate of some VLPO neurons. Interestingly, at 2 μM, propofol induced a sufficiently large depolarization that firing was inactivated. A possible explanation is that the indirect potentiation of glutamate release produced by propofol is especially pronounced in slices, where many more glutamatergic inputs to VLPO neurons are likely to be preserved.

In conclusion, our results show that chloride channel and $GABA_A$ receptors exist on the glutamatergic axons/terminals that make synapses on the VLPO neurons. By enhancing Cl^- efflux from these axons, propofol depolarizes these terminals and stimulates the release of glutamate, which increases the activity of VLPO neurons and thus potentiates GABAergic inhibition of arousal systems. This indirect mode of propofol action can be expected to contribute to its known effectiveness as a general anesthetic.

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