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The Mechanism of Inhibitory Actions of Propofol on Rat Supraoptic Neurons

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Background: **In the perioperative period, plasma osmotic pressure, systemic blood pressure, and blood volume often change dramatically. Arginine vasopressin is a key factor in the regulation of these parameters. This study was performed to evaluate the direct effects and the mechanism of the actions of propofol on arginine vasopressin release from magnocellular neurosecretory neurons in the rat supraoptic nucleus.**

Methods: **Somatodendritic arginine vasopressin release from supraoptic nucleus slice preparations was measured by radioimmunoassay. Ionic currents were measured using the whole-cell mode of the patch-clamp technique in supraoptic nucleus slice preparations or in single dissociated supraoptic nucleus neurons of the rat.**

Results: **Propofol at concentrations greater than 10^{-5} M inhibited the arginine vasopressin release stimulated by potassium chloride (50 mM). This inhibition by propofol was not reversed by picrotoxin, a gamma-aminobutyric acid$_A$ ($GABA_A$) receptor antagonist, whereas arginine vasopressin release induced by glutamate (10^{-3} M) was also inhibited by propofol at a clinically relevant concentration (10^{-6} M). The latter effect was reversed by picrotoxin. Propofol evoked Cl^- currents at concentrations ranging 10^{-6} to 10^{-4} M. Propofol (10^{-6} M) enhanced the GABA (10^{-6} M)-induced current synergistically. Moreover, propofol (10^{-6} M) prolonged the time constant of spontaneous GABA-mediated inhibitory postsynaptic currents. Furthermore, propofol (10^{-5} M and 10^{-4} M) reversibly inhibited voltage-gated Ca^{2+} currents, whereas it did not affect currents induced by glutamate (10^{-3} M).**

Conclusions: **Propofol inhibits somatodendritic arginine vasopressin release from the supraoptic nucleus, and the enhancement of GABAergic inhibitory synaptic inputs and the inhibition of voltage-gated Ca^{2+} entry are involved in the inhibition of arginine vasopressin release. (Key words: Anesthesia mechanisms; intravenous anesthetics; ion channels.)**

THE release of pituitary hormones such as arginine vasopressin (AVP) is enhanced during various noxious stimuli.[1] For example, it has been reported that the plasma AVP concentration increased during surgery[2] and that the increase was not suppressed by halothane or a high dose of morphine anesthesia.[3] In the perioperative period, plasma osmotic pressure, systemic blood pressure, and blood volume often change dramatically. These parameters are known to affect the release of AVP[4,5] by modulating electric activities of magnocellular neurons in the supraoptic and paraventricular nuclei (SON and PVN, respectively) of the hypothalamus,[6-9] which synthesize AVP and release it from the posterior pituitary into the general circulation.[10]

Previous electrophysiologic studies have revealed that magnocellular neurons in the SON and PVN are controlled by various neurotransmitters or modulators such as glutamate,[11] gamma-aminobutyric acid (GABA),[12-14] opioids,[15] biogenic amines, and peptides.[10] However, the effects of general anesthetics on these neurons have not been studied.

Gamma-aminobutyric acid is the major inhibitory transmitter regulating magnocellular neurons in the SON and PVN as other neurons in the central nervous system. GABA

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immunoreactivity has been reported in nerve endings making direct synaptic contact with magnocellular neurons in the SON and PVN, and $GABA_A$ receptors are functionally expressed in these neurons.[16,17] On the other hand, various general anesthetics enhance ionic currents carried by the $GABA_A$ receptor-Cl^- channel complex.[18,19] In the SON and PVN, GABA plays an important role in regulating AVP release acting through $GABA_A$ receptors,[13] thus suggesting that anesthetics may affect AVP release through interaction with $GABA_A$ receptors in these nuclei.

During osmotic or other stress, AVP is released not only in nerve terminals in the posterior pituitary gland but also in the soma or dendrites in the SON and PVN.[20] Several lines of evidence indicate that the somatodendritic release of these peptides plays an important role in regulating activities of neurons in the SON and PVN through positive feedback mechanisms.[21-23]

The principal purpose of the current study is to elucidate the effects and the mechanism of the actions of propofol, an intravenous anesthetic used widely in various clinical situations, on neurosecretory cells in the hypothalamus. For this purpose, we used slice preparations of the SON and evaluated the effects of propofol on somatodendritic AVP release from the SON. We further studied the mechanism of the effects of propofol by measuring ionic currents of SON neurons using the whole-cell mode of the patch-clamp technique.

Materials and Methods

Supraoptic Nucleus Slice Preparation

Experimental protocols were approved by the departmental Committee for Animal Experiments. Young adult male Wistar rats (weight, 100–200 g) were stunned by a blow to the back of the neck and rapidly decapitated. Brain slices containing the SON were cut as previously described.[24] Briefly, the brains were quickly removed and cooled at 4°C for approximately 1 min in a modified Krebs–Henseleit solution containing 124 mM NaCl, 5 mM KCl, 1.3 mM $MgSO_4$, 1.24 mM KH_2PO_4, 2 mM $CaCl_2$, 25.9 mM $NaHCO_3$, and 10 mM glucose, which was oxygenated continuously with a mixture of 95% oxygen and 5% carbon dioxide. A block containing the hypothalamus was cut from the brain and glued to the stage of a vibratome-type slicer (DSK-2000; Dosaka EM Co., Kyoto, Japan). Coronal hypothalamic slices containing the SON (400 μm thick) were cut from the block at 4°C. The slices were trimmed carefully using a circular puncher (1.8 mm ID) and preincubated in the perfusion medium (Krebs–Henseleit solution) at room temperature. They were left in the medium for at least 30 min until they were used.

Measurement of Arginine Vasopressin Release

Supraoptic nucleus slice preparations were transferred into a small perfusion chamber (volume of 200 μl) and perfused continuously with a peristaltic pump at a flow rate of 1.5 ml/min at room temperature. Experiments were begun after a 30-min equilibration period. Perfusion medium containing chemicals at various concentrations was applied to the slices from separate storage bottles. The perfusates were collected before and during stimulation for 2 min. Arginine vasopressin release in response to a drug was expressed as a percentage of basal AVP release measured just before the drug application. The AVP concentration in the perfusates were assayed by radioimmunoassay (Mitsubishi Chemical Corp., Tokyo, Japan) as previously described.[25] The minimum AVP concentration detectable in the radioimmunoassay was 0.2 pg/ml.

Dissociation of Supraoptic Nucleus Neurosecretory Cells

Supraoptic nucleus neurons were dissociated by a modified enzymatic digestion method described elsewhere.[26] Briefly, SON slices were incubated in Krebs–Henseleit solution containing trypsin for 90 min at 28°C. The slices were mechanically dissociated by trituration with fire-polished glass pipettes. The purity of the dissociated cells was examined by immunocytochemical methods described previously[27] using AVP and oxytocin antibodies (Incstar, Stillwater, MN), and it was confirmed that all cells with a surface area greater than 200 μm^2 were positively stained with the antibodies.

Whole-cell Patch Clamp of Single Supraoptic Nucleus Neurons

Dissociated cells were plated in a culture dish. The electrodes were made with a puller (P-87 Sutter Instrument Co., Novato, CA) from thick-wall borosilicate glass (GD-1.5; Narishige, Tokyo, Japan). They had a final resistance between 3 and 6 MΩ when filled with the electrode solution. The solution used for the experiments with dissociated neurons was HEPES-buffered solution containing 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 11.1 mM glucose, and 10 mM HEPES (pH 7.4 adjusted with NaOH), which was oxygenated continuously with 100% oxygen gas. The volume of the recording chamber was 1 ml, and the flow rate of the perfusion medium was 1.5 ml/min. The solution level was kept constant by a low-pressure aspiration sys-

tem. Electrophysiologic recordings were made at room temperature (23°C). Whole-cell tight-seal recordings were made from microscopically identified cells. Membrane currents were recorded using a patch-clamp amplifier (Axo-Patch 200A; Axon Instruments, Foster City, CA) and were digitized using pCLAMP software (version 6.0.3, Axon Instruments) for subsequent off-line analysis. Data were analyzed using AxoGraph software (version 3.5, Axon Instruments).

The Cl^- currents were recorded at a holding potential of −60 mV. The current-voltage relation of Cl^- currents was measured using a ramp pulse of 200 ms from −80 mV to 20 mV. To block K^+ channels, we used the pipette solution containing 110 mM CsCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM TEA-Cl, 5 mM 4-AP, 10 mM EGTA, 10 mM HEPES, and 4 mM Na_2-GTP and Mg-ATP (pH 7.2 adjusted with Tris base). Furthermore, 100 μM Cd, 100 μM Ni, and 1 μM tetrodotoxin were added to bath (HEPES-buffered solution) to block voltage-dependent Ca^{2+} channels and Na^+ channels. In some experiments, Cs-methansulfonate was used instead of CsCl. Rapid application of drugs was performed according to the "y-tube" method reported previously.[28]

Voltage-dependent Ca^{2+} currents were elicited by voltage steps from the holding potential of −80 mV to various depolarized test potentials (−60 to +20 mV). Leak currents and capacitive currents were canceled by off-line subtraction of Cd^{2+} (200 μM)-insensitive currents. The pipette solution used for Ca^{2+} current measurements contained 100 mM TEA-Cl^-, 5 mM 4-AP, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, 10 mM HEPES, 0.3 mM Na_2-GTP, 4 mM Mg-ATP, and 20 mM creatine phosphate di-tris (pH 7.2 adjusted with Tris base). The external solution was HEPES-buffered solution with 1 μM tetrodotoxin and 100 μM picrotoxin. Because Ca^{2+} currents showed "rundown," the magnitude of Ca^{2+} current inhibition was evaluated as a percentage of inhibition of the mean total currents that were measured just before and after application of drugs. Various concentrations of propofol were added to the cells by changing the bath solution with a peristaltic pump.

Measurement of Spontaneous Inhibitory Postsynaptic Currents in Slice Preparations

Slice patch-clamp experiments were performed as previously described.[24] Briefly, a slice was placed in a glass-bottomed chamber and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steal weight. The volume of the recording chamber was 1 ml and the perfusion rate was 1.5 ml/min. Magnocellular neurons in the SON were identified under an upright microscope (Axioscope/FS; Carl-Zeiss, Oberkochen, Germany) with Nomarski optics (×400). The pipette solution contained 140 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, and 2 mM Mg-ATP (pH 7.3 set with Tris base). Electrophysiologic recordings were made at room temperature. Whole-cell recordings were made from microscopically identified SON neurons in the upper surface layers of the slices. After gaining access to the whole cell, the series resistance was approximately 15–30 MΩ and the cell capacitance was 10–30 pF. Recordings of postsynaptic currents were begun approximately 5 min after membrane rupture, when the currents reached a steady state. Spontaneous (miniature) inhibitory postsynaptic currents (IPSCs) were recorded at a holding potential of −70 mV in the presence of the non-N-methyl-D-aspartate glutamate receptor antagonist, CNQX (10^{-5} M). Currents and voltages were recorded using an EPC-9 amplifier (HEKA, Lambrecht/pfalz, Germany). Signals were filtered at 3 kHz, digitized at 1 kHz using an analog-digital converter (MacLab/8; ADInstruments, Castle Hill, Australia), and stored on hard disks using a personal computer. To analyze synaptic currents quantitatively, only the AC components were used for analysis with a software package (AxoGraph, version 3.5). To minimize the influence of rundown of synaptic currents, the effects of drugs were normalized by the average of pre- and postcontrols obtained before and more than 10 min after drug application, respectively.

Statistical Analyses

The values are expressed as mean ± SD. Arginine vasopressin release was analyzed using a paired Student *t* test for the induced effects of potassium chloride or glutamate, and one-way analysis of variance with Scheffé F test for the inhibiting effects of propofol. The amplitude of Cl^- currents in response to various concentrations of propofol was analyzed using one-way analysis of variance with the Scheffé F test. The inhibitory effects of picrotoxin on propofol-induced currents were analyzed using the paired Student *t* test. Relative Cl^- current enhancement, inhibition of voltage-gated Ca^{2+} currents, and the effects on miniature IPSCs by propofol were analyzed using the Wilcoxon signed-rank test. We used the StatView (version 4.02; SAS Institute Inc., Cary, NC) software package for these statistical analyses. The number of neurons tested (n) is noted in each result or figure legend. Probability values < 0.01 or < 0.05 were considered significant.

Drugs

We used Diprivan (Zeneca, Osaka, Japan), the unique clinically available emulsion, as propofol. We used 10%

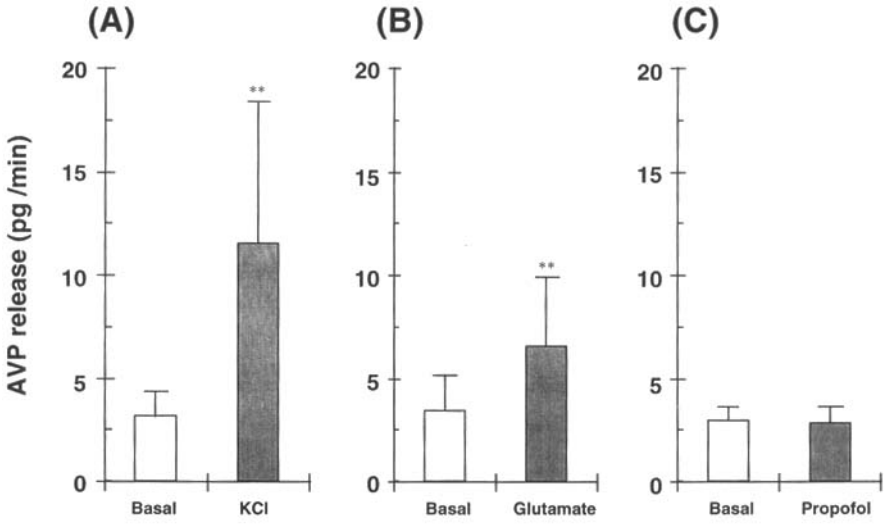

Fig. 1. The effects of potassium chloride, glutamate, and propofol on basal arginine vasopressin (AVP) release from rat supraoptic nucleus slice preparations. (*A*) Basal AVP release and AVP release stimulated by 50 mM potassium chloride for 2 min. (*B*) Basal AVP release and AVP release stimulated by 10^{-3} M glutamate for 2 min. (*C*) Basal AVP release and AVP release in response to 10^{-4} M propofol for 2 min. Results are the mean ± SD, for n = 8 (*A*), n = 10 (*B*), and n = 6 (*C*). **Significant ($P < 0.01$) by a paired Student *t* test. Potassium chloride was replaced with equimolar sodium chloride.

Intralipid (Pharmacia, Stockholm, Sweden) as the control. Tetrodotoxin was purchased from Sankyo (Tokyo, Japan); CNQX was from Sigma Chemical Company (St, Louis, MO), and other drugs were obtained from Nacalai tesque (Osaka, Japan).

Results

Effects of Propofol on Arginine Vasopressin Release

Increasing the K^+ concentration in the perfusion solution from 5 to 50 mM resulted in an increase in AVP release from SON slice preparations from 3.13 ± 1.17 pg/min to 11.48 ± 6.90 pg/min (fig. 1A). Application of glutamate (10^{-3} M) also increased AVP release from SON slice preparations from 3.37 ± 1.74 pg/min to 6.50 ± 3.33 pg/min (fig. 1B). Both increases in AVP release were significant. Propofol (10^{-4} M) had little effect on basal AVP release (fig. 1C). Propofol at 10^{-5} and 10^{-4} M caused significant reductions in the potassium chloride-evoked increase in AVP release from 359% ± 75% to 203% ± 25% and to 143% ± 23%, respectively, whereas propofol at 10^{-6} M had no significant effect (fig. 2). The

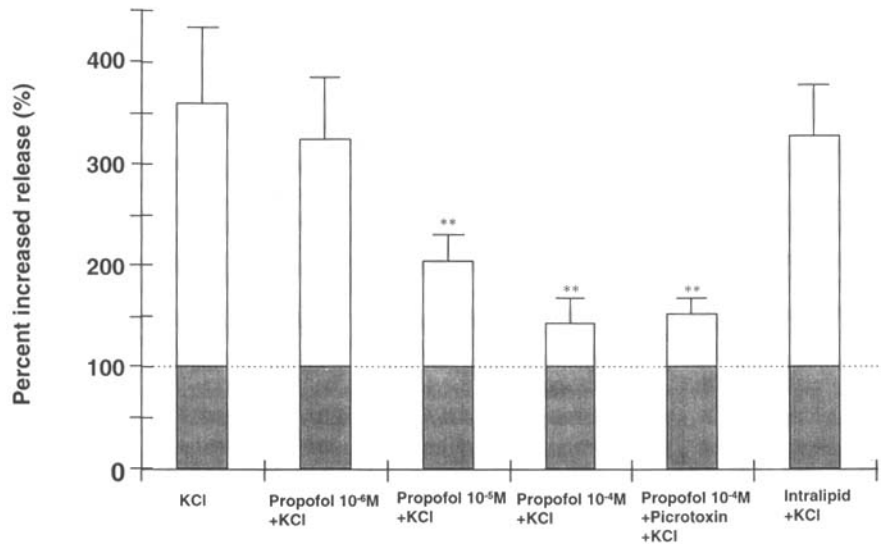

Fig. 2. The effects of propofol on somatodendritic arginine vasopressin (AVP) release stimulated by potassium chloride. The AVP release stimulated by 50 mM potassium chloride was significantly inhibited by propofol (10^{-5} M and 10^{-4} M) but was unaffected by propofol (10^{-6} M). The inhibition of potassium chloride (50 mM)–induced AVP release by propofol was not reversed by picrotoxin (10^{-4} M), a gamma-aminobutyric $acid_A$ receptor antagonist. Results are expressed as a percentage increase from basal release measured for 2 min just before potassium chloride stimulation (mean ± SD of 4–11 independent experiments). **Significant ($P < 0.01$) from the value obtained with potassium chloride only by one-way analysis of variance using the Scheffé F test. Intralipid had no effect on potassium chloride–evoked AVP release. Intralipid in this and subsequent experiments was used at 178 mg/l (the concentration equivalent to that of vehicle contained in 10^{-4} M propofol solution, if 10% Intralipid is assumed to be a vehicle of propofol).

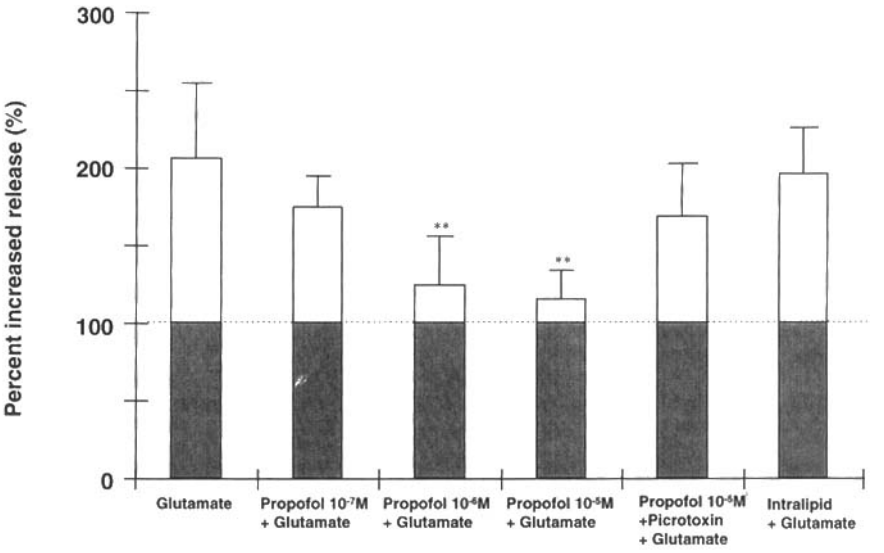

Fig. 3. The effects of propofol on somatodendritic arginine vasopressin (AVP) release stimulated by glutamate. The AVP release induced by glutamate (10^{-3} M) was inhibited by propofol at a clinically relevant concentration (10^{-6} M). The inhibition of glutamate (10^{-3} M)-induced AVP release by propofol was reversed by picrotoxin (10^{-4} M). Results are expressed as a percentage increase from basal release measured for 2 min just before glutamate stimulation (mean ± SD of 5–14 independent experiments). **Significant ($P < 0.01$) from the value obtained with glutamate only by one-way analysis of variance using the Scheffé F test. Intralipid had no effect on glutamate-evoked AVP release.

inhibition of potassium chloride-evoked AVP release by propofol (10^{-4} M) was not reversed by picrotoxin (10^{-4} M), a $GABA_A$ antagonist. Intralipid had no effect on potassium chloride-evoked AVP release. Propofol at 10^{-6} and 10^{-5} M significantly suppressed AVP release induced by glutamate (10^{-3} M) from 206% ± 48% to 124% ± 32% and to 115% ± 18%, respectively, whereas propofol at 10^{-7} M and intralipid had little effect (fig. 3). The effects of propofol (10^{-6} M) were reversed by picrotoxin (10^{-4} M).

Effect of Propofol on the Gamma-aminobutyric Acid-induced Cl^- Currents

At concentrations greater than 10^{-6} M, propofol induced inward currents in dissociated SON neurons at a holding potential of −60 mV (figs. 4A and 4B). The current voltage relation of the propofol-induced currents was evaluated by applying a ramp pulse from −80 mV to 20 mV (figs. 4C and 4D). The reversal potential of the propofol-induced currents was 0.08 ± 2.19 mV (n = 17), which was close to the reversal potential of GABA-induced currents (0.72 ± 2.25 mV, n = 9; fig. 4C). The reversal potential of the propofol-induced currents was shifted to −39.73 ± 3.77 mV (n = 14) when methansulfonate was used as the major anion in the pipette (fig. 4D). The reversal potentials calculated from the Nernst equation using Cl^- concentrations of bath solution and pipette solutions for CsCl and Cs-methansulfonate experiments are −2.18 mV and −41.6 mV, respectively. The good agreement in the two reversal potentials indicate that the propofol-induced currents were carried solely by Cl^-. Furthermore, the propofol-induced currents were blocked by picrotoxin (10^{-4} M; fig. 5). Intralipid did not induce Cl^- currents in three tests.

In the next series of experiments, the interaction between propofol and GABA was studied. Propofol at 10^{-6} M, which induced little or no inward current, enhanced the GABA (10^{-6} M)-induced Cl^- currents synergistically (fig. 6A). The enhancement by propofol (10^{-6} M) was significant when it was added to 10^{-6} M GABA. The magnitude of the enhancement by propofol was 142.07% ± 24.10% (fig. 6B). Propofol (10^{-6} M) did not enhance currents induced by GABA (10^{-5} M and 10^{-4} M), which were near maximum in amplitude. Propofol (10^{-4} M) had little or no effect on currents evoked by glutamate (10^{-3} M). The peak amplitude of the glutamate-evoked cationic currents recorded in the presence of propofol at a holding potential of −60 mV was 95.3% ± 4.8% of control (n = 3).

Effects of Propofol on the Voltage-gated Calcium Currents

Propofol caused dose-related inhibition of voltage-gated Ca^{2+} currents elicited by a voltage step to −20 mV from a holding potential of −80 mV (fig. 7A). The propofol-induced reduction in the amplitude of voltage-gated Ca^{2+} currents was rapidly reversible (fig. 7B). The current-voltage relation of the propofol-induced inhibition of Ca^{2+} currents obtained by voltage steps from −60 to 20 mV revealed that high voltage-activated currents were inhibited by propofol (fig. 7C). The propofol-induced inhibition of Ca^{2+} currents was significant at concentrations of 10^{-5} M and 10^{-4} M (fig. 7D). Intralipid had little

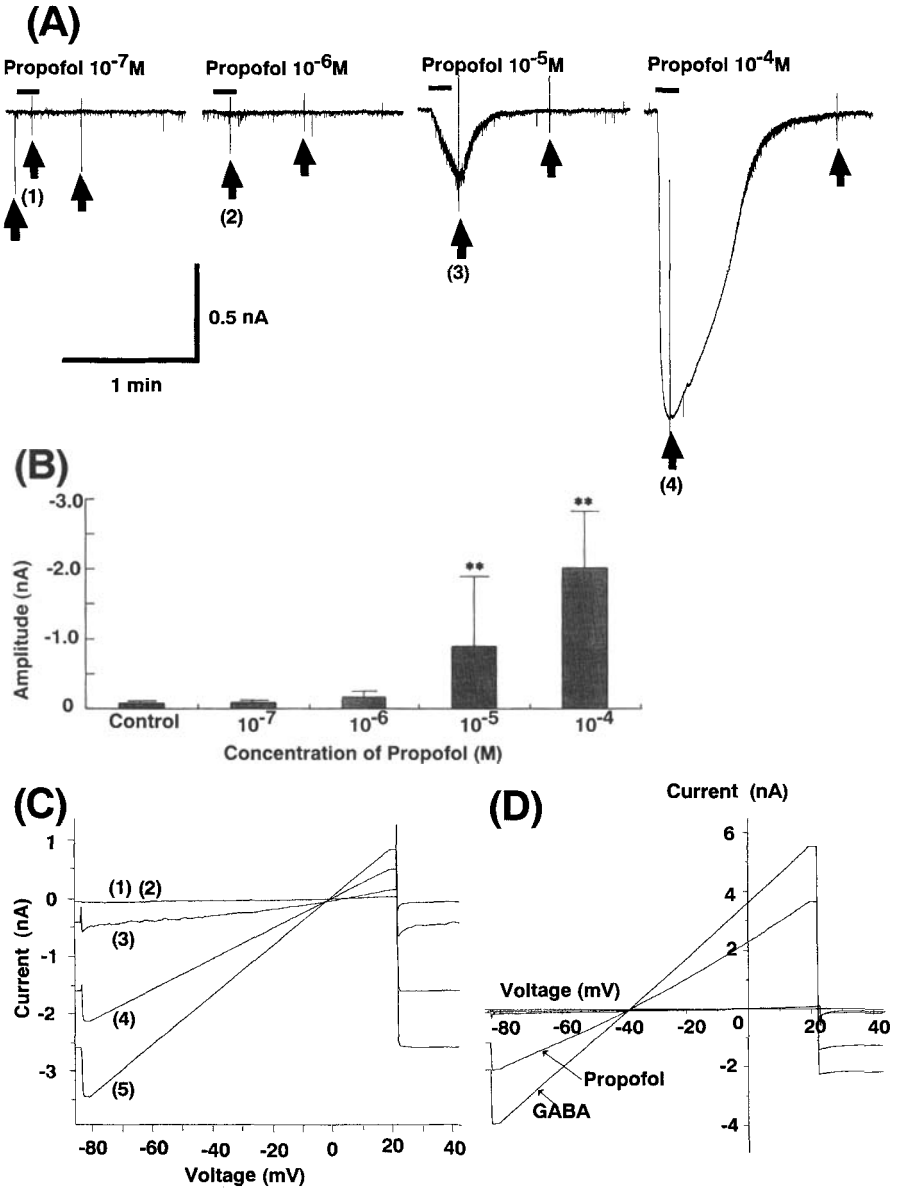

Fig. 4. Cl^- currents induced by propofol in dissociated supraoptic nucleus neurons. (*A*) Representative current traces showing dose-dependent responses to propofol. The holding potential (Vh) was −60 mV. Arrows indicate the time when ramp pulses from −80 to 20 mV (for 200 ms) were applied. (*B*) The peak amplitude of the propofol-induced currents against the concentrations of propofol. Control represents currents measured in the absence of propofol. At concentrations greater than 10^{-6} M, propofol induced significant currents. Results are the mean ± SD of nine independent experiments. **Significant ($P < 0.01$) from control by one-way analysis of variance using the Scheffé F test. (*C*) Current-voltage (I-V) relations of propofol-induced currents in response to ramp pulses from −80 to 20 mV. The I-V relations of (1)-(4) are obtained by ramp pulses shown in *A*. The I-V relation of currents induced by 10^{-4} M gamma-aminobutyric acid is also shown (5). (*D*) The I-V relations of propofol-induced currents obtained with methansulfonate as the major anion in the pipette. The I-V relations were shifted toward the left (hyperpolarized potentials).

effect on Ca^{2+} currents elicited by a voltage step to −20 mV (100.2% ± 0.2% of control; n = 3).

Effects of Propofol on Miniature Inhibitory Postsynaptic Currents

Because it has been reported that SON neurons receive massive spontaneous synaptic inputs by GABA and glutamate,[13,14] we evaluated the effects of propofol on miniature IPSCs. Propofol (10^{-7} M to 10^{-5} M) caused little effect on the amplitude and frequency of IPSCs (fig. 8), whereas it significantly prolonged the time constant of the decay phase of IPSCs at concentrations of 10^{-6} M and 10^{-5} M (fig. 8E). The time constant was increased to 113% ± 11% (n = 7) and 120.6% ± 14.8% (n = 8) of control in response to 10^{-6} M and 10^{-5} M propofol, respectively. Intralipid had little effect on the time constant (97.79% ± 4.61% of control; n = 4).

Discussion

The current study provided the first direct evidence that propofol inhibits the activity of AVP neurons in the SON, as

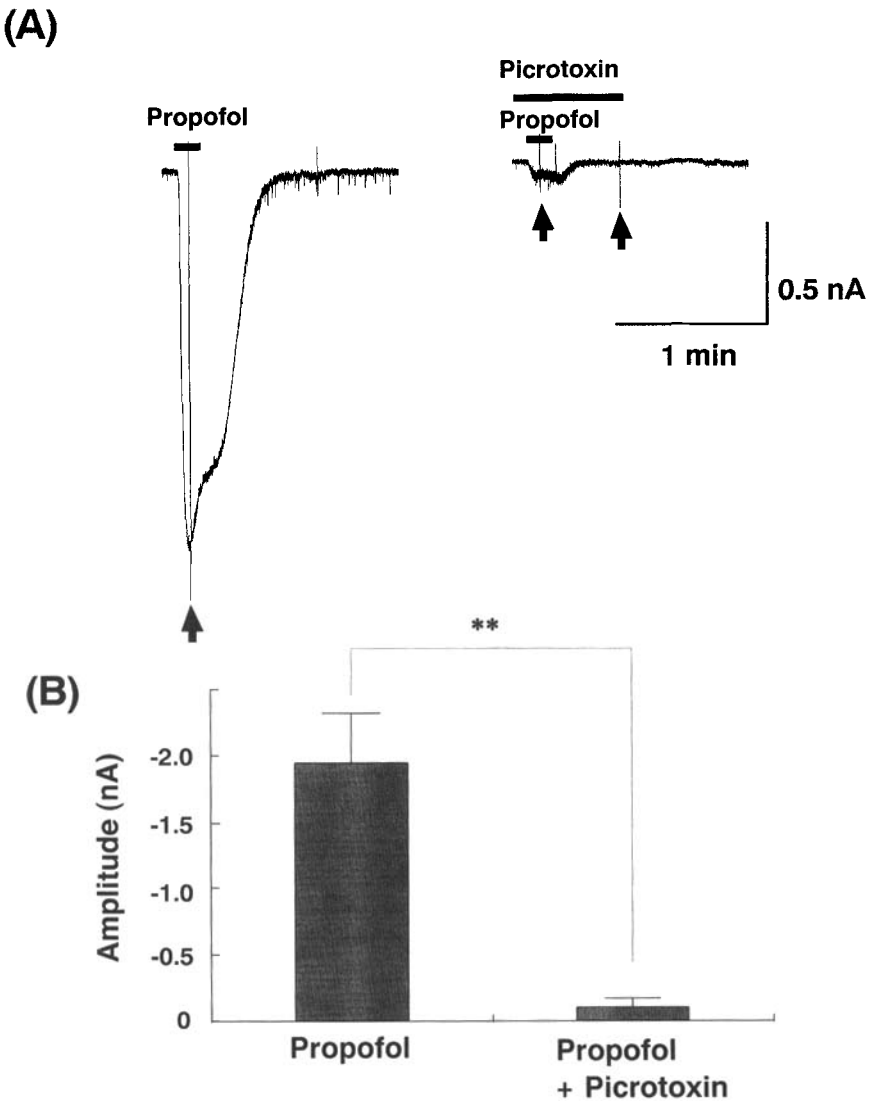

Fig. 5. The effects of picrotoxin on propofol-induced currents. (*A*) Propofol-induced (10^{-4} M) inward currents were blocked by picrotoxin (10^{-4} M). The holding potential (Vh) was −60 mV. Arrows indicate the time when ramp pulses from −80 to 20 mV (for 200 ms) were applied. (*B*) Summary data for the inhibition of propofol-induced currents by picrotoxin (n = 5). **Significant ($P < 0.01$) by the paired Student *t* test.

measured by somatodendritic AVP release from SON slice preparations. The inhibition by propofol was observed when SON slice preparations were stimulated with potassium chloride and glutamate. Stimulation with KCl produced a larger increase in AVP release than with glutamate, and 10^{-5} M or 10^{-4} M propofol was needed to reduce the increase significantly. Furthermore, the inhibition of potassium chloride–evoked AVP release by 10^{-4} M propofol was not reversed by picrotoxin, indicating that the inhibition of AVP release by this concentration of propofol is a result of effects other than $GABA_A$ receptor-mediated mechanisms. On the other hand, glutamate, a physiologically relevant excitatory transmitter for SON neurons,[17,29] caused a relatively smaller increase in AVP release, and the increase could be inhibited by a low concentration (10^{-6} M) of propofol. Because the inhibition by the low concentration of propofol was reversed by picrotoxin, it is likely that $GABA_A$ receptor-mediated mechanisms underlie the inhibition. These results indicate that at least two distinct mechanisms are involved in the inhibition of SON neurons by propofol. Then we evaluated the mechanisms of the propofol-induced inhibition using the whole-cell patch-clamp technique.

First, we studied the picrotoxin-sensitive inhibitory mechanism in the SON. Direct interaction between propofol and glutamate receptors could be excluded because we have observed that glutamate-induced currents were unaffected by propofol at concentrations as

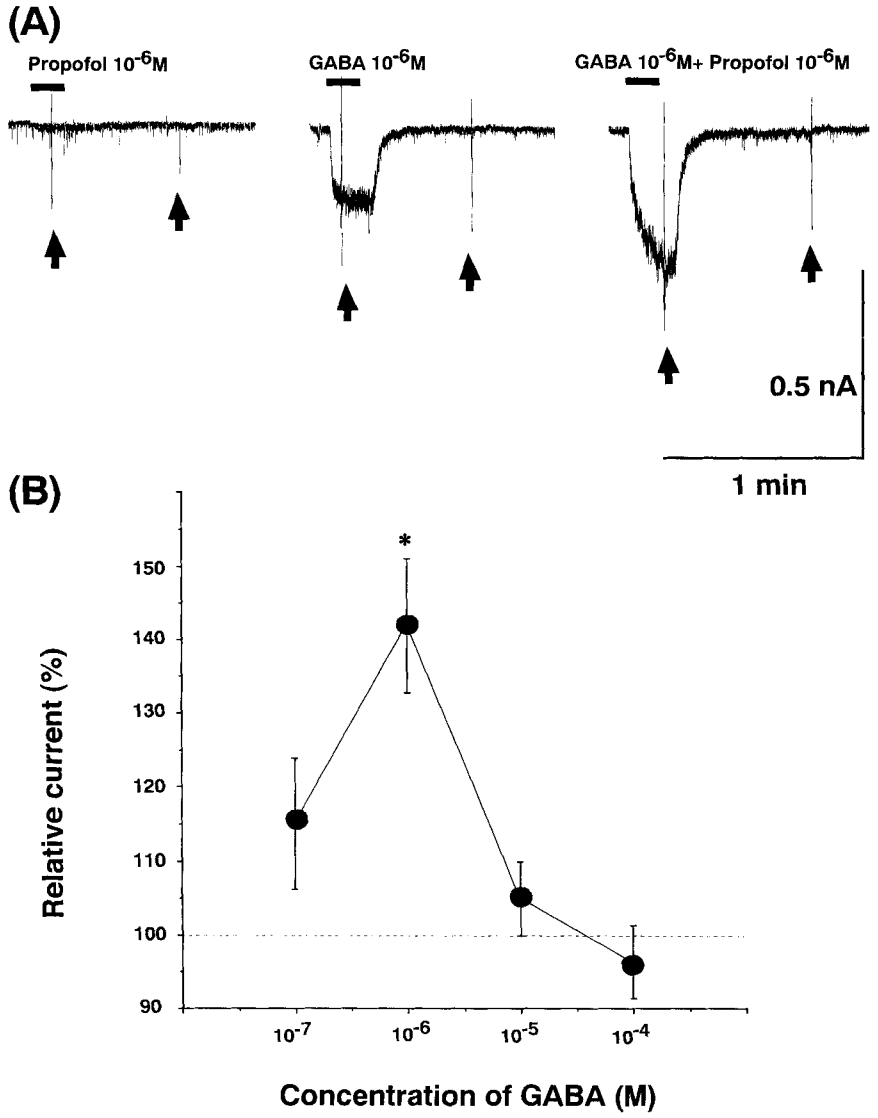

Fig. 6. The effects of 10^{-6} M propofol on the gamma-aminobutyric acid (GABA)–induced Cl^- current. (*A*) Propofol (10^{-6} M) enhanced the GABA-induced (10^{-6} M) Cl^- currents synergistically. The holding potential (Vh) was −60 mV. Arrows indicate the time when ramp pulses from −80 to 20 mV (for 200 ms) were applied. (*B*) Summary data for enhancement of Cl^- currents by 10^{-6} M propofol. Results are expressed as a percentage increase from currents obtained with GABA alone (the mean ± SD of seven experiments). *Significant ($P < 0.05$) by the Wilcoxon signed-rank test.

great as 10^{-4} M. In dissociated SON neurons, propofol induced Cl^- currents at concentrations ranging from 10^{-5} M to 10^{-4} M. Because the propofol-induced currents were blocked by picrotoxin, the currents seem to be mediated by $GABA_A$ receptors. Although 10^{-6} M propofol caused little current by itself, it synergistically enhanced GABA-evoked currents. These results are consistent with previous findings that propofol enhanced GABA-induced Cl^- currents in rat cerebral cortex neurons,[30] bovine adrenomedullary chromaffin cells and rodent central neurons,[31] and rat hippocampal pyramidal neurons.[32] Propofol at lower concentrations (such as 10^{-6} M) appears to inhibit SON neurons by enhancing the inhibitory effects of endogenously released GABA. To test this hypothesis, we recorded miniature IPSCs in SON slice preparations to identify such interaction. Although propofol caused no evident effects on the amplitude and frequency of miniature IPSCs, it significantly prolonged the time constant of the decay phase of IPSCs at concentrations between 10^{-6} M and 10^{-5} M. These results are consistent with a previous report that propofol increased the decay constant of $GABA_A$ receptor-mediated IPSCs in the rat hippocampus,[33] and they are compatible with the hypothesis that propofol enhances IPSCs carried by endogenously released GABA. The lack of effect of propofol on the frequency of miniature IPSCs indicates that propofol has little effect on presynaptic terminals. Furthermore, the lack of effect of propofol on

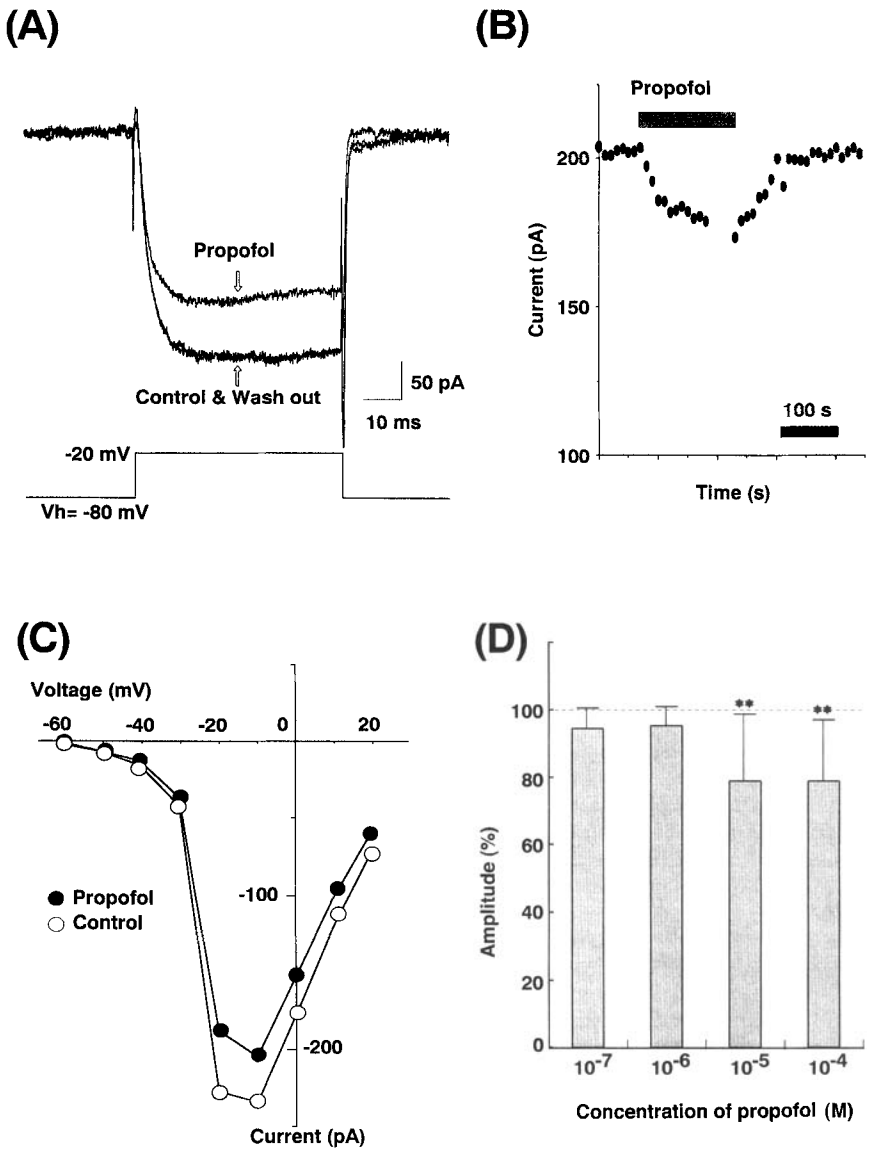

Fig. 7. The effects of propofol on voltage-gated Ca^{2+} currents. (*A*) Representative traces of Ca^{2+} currents elicited by voltage steps to −20 mV from the holding potential (Vh) of −80 mV before (control), during, and after (washout) application of 10^{-4} M propofol. Propofol reversibly inhibited voltage-gated Ca^{2+} currents. (*B*) Representative time course of propofol-induced inhibition of Ca^{2+} currents. Voltage-step commands to −20 mV from −80 mV were applied every 10 s, and the peak Ca^{2+} currents are plotted against time. (*C*) The current-voltage relation of Ca^{2+} currents recorded before and during application of 10^{-4} M propofol. (*D*) The dose-response relation of the propofol-induced inhibition of the Ca^{2+} current. The data are shown as a percentage of the total currents measured just before propofol application (the mean ± SD of 10 experiments). **Significant ($P < 0.01$) by the Wilcoxon signed-rank test.

the amplitude of IPSCs suggests that GABA at synaptic cleft might reach a nearly maximum concentration around $GABA_A$ receptors in the postsynaptic membrane because we have shown that the enhancement of GABA-mediated currents by propofol was observed in a narrow GABA concentration range.

Next we sought a candidate for the picrotoxin-insensitive inhibitory mechanism in the SON. The current result that propofol produced rapidly reversible inhibition of Ca^{2+} currents in dissociated SON neurons provided one candidate. Similar inhibition of Ca^{2+} currents by propofol has been reported in porcine tracheal smooth muscle cells,[34] rat vascular smooth muscle cells,[35] and rat cardiomyocytes.[36] Because several lines of evidence suggest that Ca^{2+} influx through voltage-dependent Ca^{2+} channels during action potentials is important in somatodendritic AVP release in the SON,[26,37] the inhibition of Ca^{2+} currents may explain, at least in part, the picrotoxin-insensitive component of the propofol-induced inhibition of AVP release. Because the magnitude of the propofol-induced inhibition of Ca^{2+} currents was considerably smaller than the magnitude of the propofol-induced inhibition of K^+-evoked AVP release, mechanisms other than Ca^{2+} channel inhibition also could be involved in the picrotoxin-insensitive inhibition of AVP release. It has been reported that at high

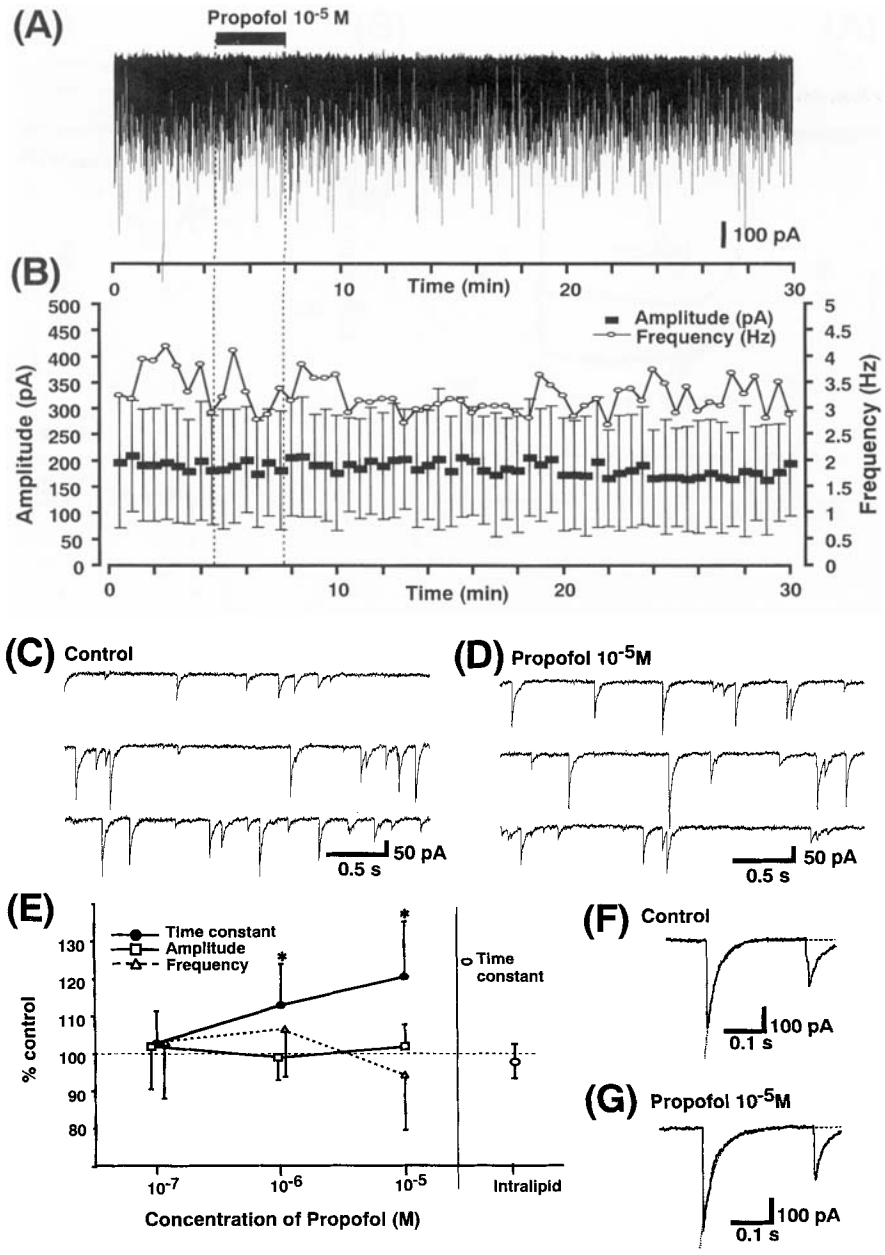

Fig. 8. The effects of propofol on miniature inhibitory postsynaptic currents (IPSCs) recorded in supraoptic nucleus slice preparations. (*A*) A representative trace of IPSCs shows a response to 10^{-5} M propofol. The holding potential was −70 mV. 6-cyano-7-nitroquinoxaline-2,3-dione (10^{-5} M) was added to block miniature excitatory postsynaptic currents. The solid horizontal bar indicates the time when 10^{-5} M propofol was applied. (*B*) The time course of changes in the amplitude and frequency of IPSCs shown in *A*. The frequency was determined every 30 s using the peak detection function in Axograph software, version 3.5 (Axon Instruments). The amplitude was an average of the peak currents detected during each 30 s, and the error bars are the SD of the amplitude. The noise level was set as −10 pA and the volley level was set as 50% of the preceding peak. (*C* and *D*) Representative traces of IPSCs recorded before and during application of 10^{-5} M propofol. (*E*) Dose–response relations of the effects of propofol on the time constant, amplitude, and frequency of miniature IPSCs obtained from 5 (10^{-7} M), 7 (10^{-6} M), 8 (10^{-5} M), and 4 (Intralipid) neurons. The time constant of the decay phase was calculated using a single exponential fit from 17 to 25 synaptic currents randomly selected in each neuron. All three parameters are shown as a percentage change from the average of pre- and postcontrols obtained before and more than 10 min after propofol application. *Significant ($P < 0.05$) by the Wilcoxon signed-rank test. (*F* and *G*) Examples of single exponential fit (*dotted line*) for IPSCs recorded before and during application of 10^{-5} M propofol.

concentrations, propofol caused nonselective inhibition of other ion channels, such as Na^+ and K^+ channels.[38,39] These mechanisms or unidentified inhibitory mechanisms downstream of the ion channels could explain the large inhibition of K^+-evoked AVP release that we observed in the current study. Alternatively, propofol could preferentially inhibit Ca^{2+} channels, which play predominant roles in somatodendritic AVP release. The current-voltage relation of propofol-induced inhibition indicates that high-threshold Ca^{2+} channels receive an inhibitory influence by propofol, and four distinct subtypes (L, N, P/Q, and R) of high-threshold Ca^{2+} currents have been identified in SON neurons.[40,41]

The current results indicate that in SON neurons, propofol activates $GABA_A$ receptor Cl^- channels at concentrations of 10^{-6} M or greater and inhibits voltage-gated calcium channels at concentrations of 10^{-5} M or more. Although estimating effective concentrations in the brain and plasma in clinical situations of intravenous anesthetics is difficult because of their lipid solubility

and interaction with plasma proteins, previous reports suggested that clinically relevant concentrations of propofol in such *in vitro* experiments would be approximately 10^{-6} M,[42-46] which is closer to the concentration of propofol that inhibited glutamate-evoked AVP release and enhanced GABA-induced Cl^- currents in SON neurons in the current study.

In the current study we measured AVP release from the SON but not from the posterior pituitary. Therefore, it is not clear whether the inhibition by propofol actually suppresses AVP release into the systemic circulation. However, the current results could be extrapolated to the effects of propofol on plasma AVP levels, because it has been reported that the activity of magnocellular neurons in the SON and PVN is closely correlated with the plasma AVP concentration.[21-23]

In conclusion, a clinically relevant concentration of propofol inhibited somatodendritic AVP release in the SON stimulated by glutamate by enhancing inhibitory effects of endogenously released GABA through a postsynaptic mechanism. Furthermore, higher concentrations of propofol suppressed somatodendritic AVP release induced by high K^+ by inhibiting voltage-gated Ca^{2+} currents. Because SON neurons are known as one of the final common output pathways from the central nervous system, the approach that we have used in the current study may provide a good model to analyze the mechanism of regulation of central nervous system neurons by general anesthetics.

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