Propofol Activates GABA<sub>A</sub> Receptor-Chloride Ionophore Complex in Dissociated Hippocampal Pyramidal Neurons of the Rat

Manami Hara, D.D.S.,* Yoshihisa Kai, D.D.S.,† Yoshimi Ikemoto, M.D.‡

**Background:** The molecular mechanism of propofol anesthesia has been related to facilitation of the inhibitory neurotransmission mediated by γ-aminobutyric acid (GABA). In the current study, the authors examined the direct actions of propofol on the acutely dissociated mammalian central neurons.

**Methods:** Hippocampal pyramidal neurons were dissociated after enzymatic treatment of the brain slices of the rat. Single neurons were voltage-clamped using the whole cell configuration of the patch clamp technique, and drugs were applied with a rapid drug-application system.

**Results:** In the pyramidal neurons voltage-clamped at ~60 mV, propofol evoked a transmembrane inward current, which desensitized at high concentrations of the anesthetic. The peak amplitude of the current increased sigmoidally with increasing doses of propofol applied. A least-squares fitting gave a dissociation constant of 1.2 × 10<sup>−7</sup> M and a Hill coefficient of 1.8, thereby indicating that clinical concentrations of propofol evoke the current, and that the anesthetic cooperatively activates the channel. The threshold concentration of propofol was less than 10<sup>−6</sup> M. The reversal potential for the current shifted according to the chloride equilibrium potential predicted by the Nerst equation, indicating that the current was carried by chloride ions. Bicuculline and strychnine suppressed the current in a concentration-dependent manner, in which the former was almost 40-fold more potent than the latter. The propofol-induced current desensitized with the GABA<sub>A</sub>-induced current, but no such interaction was observed with the glycine-induced current. Ro15-1788 (10<sup>−6</sup> M), an allosteric benzodiazepine antagonist, had no effect on the response. Diazepam (10<sup>−4</sup> M) enhanced the propofol-induced current, but pentobarbital (10<sup>−4</sup> M and 3 × 10<sup>−5</sup> M) did not affect the current.

**Conclusions:** Propofol at clinically relevant concentrations directly activates the GABA<sub>A</sub> receptor-chloride ionophore complex in the mammalian central neurons, and, hence, increases the chloride conductance, which may contribute to anesthesia produced by the anesthetic. The desensitization of the GABA<sub>A</sub> receptor in the presence of high concentrations of propofol may result in a suppression of the GABA<sub>A</sub> inhibitory system. (Key words: Anesthetics, intravenous; propofol. Brain: hippocampus. Ions: chloride. Measurement techniques: patch clamp. Receptors: GABA<sub>A</sub>.)

In experimental studies in vivo and in vitro, investigators have suggested an enhancement by propofol of the inhibitory neurotransmission produced by γ-aminobutyric acid (GABA) in the central nervous system. Similarly, the mechanism of anesthetic action of several drugs, including volatile general anesthetics, barbiturates, benzodiazepines, steroids, and etomidate, has been attributed to the augmentation of the GABA<sub>A</sub>-induced inhibition. In the current experiments, we studied the direct postsynaptic effects of propofol on dissociated mammalian central neurons, and found that the hydrophobic intravenous anesthetic directly activates the GABA<sub>A</sub> receptor-ionophore complex and evokes a chloride current, which results in suppression of the neuronal excitability of the central nervous system.

**Materials and Methods**

**Preparation**

The pyramidal neurons in the hippocampal CA1 region were acutely dissociated from 1- to 2-week-old Wistar rats using a technique similar to that developed by Kaneda et al. In brief, the rats were decapitated under ether anesthesia and the brain was rapidly dissected and sliced 400 µm thick with a microslicer (Do-
saka DTK-1000, Kyoto, Japan) in an ice-cold physiologic salt solution (PSS, see below). After preincubation in the PSS for 50 min at room temperature, the slices were treated first with 0.01% Pronase (Protease, Streptomyces griseus, Calbiochem, La Jolla, CA), and then with 0.01% protease Type X (Sigma, St. Louis, MO) at 31°C for 20 min each. Thereafter, the slices were kept in the PSS at room temperature for up to several hours. Solutions were continuously oxygenated throughout the procedures. The hippocampal CA1 region was micropunched out from the slices with a small needle tip. Then, the neurons were mechanically dissociated in a culture dish by pipetting gently (namely, repeated uptake and expulsion of a cluster of neurons) with fire-polished glass pipettes. After dissociation, the neurons settled on the bottom of the dish in 30 min.

Electrical Measurements
Electrical measurements were performed using the whole cell configuration of the patch clamp technique. The resistance between the recording patch pipette and the reference electrode was 4–8 MΩ. The transmembrane currents were recorded with a patch clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, CA) and monitored on both a storage oscilloscope (Textronix 5113, Beaverton, OR) and a linear recorder (Graphitec WR3500, Tokyo, Japan), and stored on video tapes after digitization with a digital audio processor (Sony PCM-501ES, Tokyo, Japan; 16-bit resolution and altered for DC to 20 KHz).

Application of Drugs
Because of desensitization of the receptor-ionophore complex, slow bath application of agonists may obscure the precise peak value of the response. We, therefore, adopted a rapid application technique described as the "Y-tube" method which enables complete exchange of the external solution surrounding the neuron within 10 ms, so that the accurate peak value of the response can be obtained before desensitization develops. An interval of 5 min was usually allowed between applications of drugs.

Solutions
The ionic composition of the external solution (PSS) was (in mm): NaCl 150, KCl 5, CaCl₂ 2, MgCl₂ 0.5, glucose 10, and HEPES 10. The pH was adjusted to 7.4 with Trizma-base. The intrapipette solution composed of (in mm): KCl 150, MgCl₂ 2, Na₂ATP 2, EGTA 5, CaCl₂ 0.25, and HEPES 10, and the pH was adjusted to 7.2 with Trizma-base. In some experiments, KCl was partly replaced with K-gluconate to change the equilibrium potential for chloride ions.

Drugs
Drugs used in the current study were as follows: propofol (2,6-diisopropylphenol; Tokyo Kasei, Tokyo, Japan), γ-aminobutyric acid (Ishizu, Osaka, Japan), glycine (Ishizu), bicuculline (Sigma), strychnine (Sigma), pentobarbital sodium (Ishizu), and dimethyl sulfoxide (Ishizu). Ro15-1788 was provided by Hoffman La Roche (Basel, Yoshitomicho, Switzerland) and diazepam by Yoshitomi (Yoshitomicho, Japan).

Propofol was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mm and diluted to appropriate concentrations in the PSS. Bicuculline, strychnine, diazepam, and Ro15-1788 were dissolved in DMSO at 10⁻² m and diluted in the PSS 1,000 times or more. The highest concentration of DMSO used in the current study was 0.4%, which had no effects on the current in separate experiments. All other drugs were dissolved directly in the PSS. All experiments were carried out at room temperature around 22°C. Data are expressed as mean ± SD, and Student’s t test was employed to estimate the significance when appropriate. These studies were approved by the departmental Committee for Animal Experiments.

Results
Propofol Induces a Chloride Current
Propofol (1 to 200 × 10⁻⁶ M) evoked a transmembrane inward current in the pyramidal neurons voltage-clamped at −60 mV (fig. 1A). The threshold concentration was less than 10⁻⁶ M. At higher concentrations of propofol, the current waned in the continuous presence of the agent, probably because of desensitization. The rate of decline became greater with increasing concentrations of the agent. The relationship between the peak amplitude of the current and the concentration of propofol is illustrated in figure 1B, in which all responses are normalized to the peak amplitude induced by 2 × 10⁻⁵ m of the anesthetic. The concentration-response relation was sigmoidal and in accord with the conventional expression:

\[ I = I_{\text{max}} \cdot C^n / (C^n + K_c^n), \]

where \( I \) = the observed propofol-induced current; \( I_{\text{max}} \) = the maximum value of the current; \( C \) = the propofol
ACTIVATION BY PROPOFOL OF GABA<sub>A</sub> RECEPTOR CHANNEL

**Propofol Activates the GABA<sub>A</sub> Receptor-Ionophore Complex**

The effects of two conventional blockers on the current are shown in figure 3. The current traces in figure 3A were obtained in a same single neuron voltage-clamped at −60 mV. Bicuculline (BIC), a GABA<sub>A</sub> antagonist, had a greater depressant effect than strychnine (STR), a glycine (Gly) antagonist. Figure 3B illustrates the dose-inhibition relationships of BIC and STR, showing that both inhibitors suppressed the current in a dose-dependent fashion. The data points were fitted according to the equation:

\[
I = I_{\text{max}} \cdot \left( 1 - C^n / (C^n + K_D^n) \right)
\]

where \( C \) = the concentration and \( K_D \) = the dissociation constant of the blocker. A least-squares fitting for the BIC inhibition gave a \( K_D \) of \( 1.1 \times 10^{-6} \) M and a Hill coefficient of 0.9. For the STR inhibition, only a rough estimation could be made because of the lack of data points below 0.5 on the ordinate, providing a \( K_D \) of \( 4.2 \times 10^{-5} \) M and a Hill coefficient of 0.8. The continuous lines were drawn using those values and \( I_{\text{max}} \) of 1. The result shows that BIC is almost 40 times more potent than STR for the inhibition, thereby indicating that the propofol-induced chloride current flows through the GABA<sub>A</sub> receptor-chloride ionophore complex.

As depicted in figure 4A, both \( 10^{-4} \) M GABA and \( 10^{-4} \) M propofol, evoked a large desensitizing current. When propofol was applied immediately after \( 10^{-4} \) M GABA, propofol failed to evoke the current. Similarly, GABA immediately after propofol did not induce the current either (not shown). This result means that the cross-desensitization between GABA and propofol-induced responses occurred. A simultaneous application of a saturating concentration (\( 10^{-4} \) M, see figure 1 in the current study for pro-

---

**Fig. 1.** Transmembrane currents evoked by propofol in dissociated pyramidal neurons of the rat hippocampus. (A) Various concentrations of propofol were applied to a single dissociated neuron voltage-clamped at a holding potential of −60 mV. The anesthetic evoked an inward current at concentrations between \( 10^{-5} \) and \( 2 \times 10^{-4} \) M. The current declined in the continuous presence of higher concentrations of the agent, and the decline became faster depending on the concentration. Each horizontal bar above the trace indicates the period of a continuous application of propofol. (B) The concentration-response relationship of propofol. The peak amplitude of the currents was normalized to that evoked by \( 2 \times 10^{-3} \) M of the anesthetic (*) and plotted against the propofol concentration. A least-squares fit was performed using equation 1, and gave a \( K_D \) of \( 1.2 \times 10^{-3} \) M and a Hill coefficient of 1.8. Each point is the mean of five to seven experiments, and the vertical bar indicates one SD.

concentration; \( K_D \) = the dissociation constant; and \( n \) = the Hill coefficient. A least-squares fitting gave a \( K_D \) of \( 1.2 \times 10^{-5} \) M and a Hill coefficient of 1.8. The continuous line in figure 1B was drawn using those values and \( I_{\text{max}} \) of 1.42.

Figure 2A depicts the currents evoked by \( 2 \times 10^{-5} \) M propofol at various holding potentials. The current was inward at negative potentials and outward at positive potentials. In figure 2B, the peak amplitudes were normalized to that evoked at −60 mV and plotted against the membrane potential. The current-voltage relation was almost linear, and the current reversed its polarity at about 3 mV, which is close to the calculated equilibrium potential for chloride ions (0.9 mV) in the current condition. When the chloride ion concentration in the pipette was reduced to 40 mm, the current reversed its polarity at about −30 mV (not shown), which is also close to the equilibrium potential for chloride ions predicted by the Nernst equation. These findings indicate that the chloride ion is responsible for the membrane current evoked by propofol.
response as that evoked by a single application of propofol, and *vice versa.*

**Some Pharmacologic Properties of the Propofol Response**

It is known that diazepam and pentobarbital interact allosterically with the GABA receptor channel complex to augment the chloride current. A GABA-mimetic action of high concentrations of pentobarbital was shown in the amphibian and mammalian neurons. In the current experiments, diazepam or Ro15-1788 at a concentration of $10^{-6}$ M, or $10^{-5}$ and $3 \times 10^{-6}$ M of pentobarbital, did not elicit a detectable membrane current

---

**Fig. 2.** The current-voltage relationship of the propofol-induced current. *(A)* Propofol ($2 \times 10^{-6}$ M) was applied to the single neurons voltage-clamped at various holding potentials ranging from −60 to 40 mV. The current was inward at negative potentials and outward at positive potentials. *(B)* The current-voltage relation was almost linear for the propofol-induced response. The continuous line was drawn by eye. The current reversed its polarity at about 3 mV, which is close to the equilibrium potential for chloride ions predicted by the Nernst equation (0.9 mV). All currents were normalized to that evoked at −60 mV (*c*). Each point shows the mean of four to six experiments, and the bar indicates one SD.

---

**Fig. 3.** Effects of BIC and STR on the propofol-induced current. *(A)* An inward current was evoked by $2 \times 10^{-6}$ M propofol in a single neuron at −60 mV (*a* and *c*). Simultaneously applied BIC (*b*) suppressed the response more markedly than STR (*d*) at the same concentration ($10^{-5}$ M). *(B)* The concentration-inhibition curves for BIC and STR on the $2 \times 10^{-6}$ M propofol-induced current. The ratio of the currents in the presence and absence of the inhibitors is plotted against the concentration of the agents. A least-squares fit according to equation 2 yielded a $K_{0.5}$ of $1.1 \times 10^{-6}$ M and a Hill coefficient of 0.9. A rough estimation for the STR effect gave a $K_{0.5}$ of $4.2 \times 10^{-5}$ M and a Hill coefficient of 0.8. Each point is the mean of five to six neurons, and the vertical bar indicates one SD.
in neurons voltage clamped at -60 mV (not shown). Ro15-1788 (10^{-6} M), an antagonist of the benzodiazepine regulatory site, did not suppress the propofol-induced current (fig. 5A). Diazepam (10^{-6} M) significantly (P < 0.05, n = 4) potentiated the propofol response to 1.4 ± 0.26-fold of the control level. However, pentobarbital (10^{-6} M) did not augment or suppress the propofol response (fig. 5C). A higher concentration of the agent (3 × 10^{-5} M) did not affect the response either (not shown).

Discussion

Activation of the GABA<sub>A</sub> Receptor-Chloride Ionophore Complex by Propofol

For the molecular mechanism of the action of propofol in the central nervous system, experimental data have been collected to demonstrate an enhancement of the synaptic inhibition mediated by GABA. In the current experiments with dissociated hippocampal CA1 pyramidal neurons of the rat, we have shown that propofol itself induced a transmembrane current. The concentration dependence of the rate of decline of the current (fig. 1A) is consistent with desensitization of other ligand-gated currents induced by GABA, acetylcholine, or glutamate, indicating that propofol activated a receptor-operated channel. The current reversed its polarity according to the equilibrium potential for chloride ions (fig. 2), indicating that the chloride ion is the charge carrier of the current. This finding agrees with the results of Hales and Lambert that propofol applied locally by pressure ejection evoked a chloride current in bovine adrenomedullary chromaffin cells. Bath application of 8.4–252 × 10^{-6} M of the anesthetic in their experiments also evoked a current, although the peak was blunt by desensitization. The increase in the chloride conductance leads to suppression of the neuronal activity by clamping the membrane voltage at the chloride equilibrium potential, which is fairly negative (approximately -80 mV) because of low concentrations of intracellular chloride ions (3–5 mM).

The current was suppressed by BIC more extensively than by STR (fig. 3), thereby indicating that the chloride current flowed through the GABA-receptor ionophore complex. This notion was confirmed by the cross-desensitization between GABA and propofol responses (fig. 4A), and by the absence of summation of the two responses induced by a saturating concentration of the agents (fig. 4B). Gly, another inhibitory neurotransmitter in the central nervous system and known to activate a chloride channel, did not cross-desensitize with the propofol-induced current in our study (fig. 4C), reflecting no activation of the Gly receptor channel by propofol. Direct activation of the GABA<sub>A</sub> receptor-ionophore complex has also been reported with other drugs, such as chlormethiazole, volatile anesthetic-
Fig. 5. Pharmacologic characteristics of the propofol-induced current at $-60$ mV. (A) Propofol (10$^{-3}$ M) evoked an inward current at $-60$ mV (a). Simultaneously applied Ro15-1788 (10$^{-6}$ M) did not affect the propofol-induced current (b). (B) A facilitatory effect of diazepam (10$^{-6}$ M) was observed on the propofol response. The increase in the current was significant ($P < 0.05$, $n = 4$). (C) Pentobarbital (PB; 10$^{-6}$ M) did not augment or suppress the propofol-induced current. Similar results were obtained with three other neurons.

The concentration-response curve for propofol gave a Hill coefficient of 1.8 (fig. 1B), which indicates that two or more molecules of propofol cooperatively activate the GABA$_A$ receptor channel. This is consistent with the finding of Shirasaki et al. on the GABA-activated channel in the same preparation.\textsuperscript{18} On the other hand, Levitan et al. did not notice cooperativity of GABA in the receptors that were yielded on oocytes by coexpression of $\alpha$ and $\beta$ subunit clones.\textsuperscript{30} And, in Aplysia neurons, cooperativity of GABA was not observed to activate the channel. The GABA-induced current was never enhanced, but was depressed noncompetitively by pentobarbital, and was not affected by diazepam.\textsuperscript{25} It is of interest, therefore, to know whether propofol acts cooperatively to activate the GABA channels in these preparations.

Hales and Lambert found, with single-channel recordings, that propofol (30 $\times$ 10$^{-6}$ M) activates two classes of chloride channel, with a unit conductance of 29 and 12 pS. However, GABA (10$^{-6}$ M)-activated channels had unit conductances of 33, 16, and 10 pS.\textsuperscript{23} The unit conductances of the propofol-induced current in mammalian neurons remain to be determined.

Another direct action of propofol on membrane currents was reported by Magnelli et al. in patch-clamp experiments with pheochromocytoma cells (PC 12), in which they described a dose-dependent reduction in the delayed outward potassium current by high concentrations of propofol (50 to 1,000 $\times$ 10$^{-6}$ M). They also recorded two classes of potassium single-channel currents (10 and 22 pS), showing that lower concentrations of propofol (below 140 $\times$ 10$^{-6}$ M) completely abolished the opening of the 22-pS channel with nearly no effect on the 10-pS channel. However, unphysiologically high concentrations depressed the activity of the both classes.\textsuperscript{31}

**Propofol Binding Site is Distinct From That of Benzodiazepines**

Investigators have revealed that the GABA$_A$ receptor-ionophore complex is a heterooligomer consisting of five subunits (some of $\alpha$, $\beta$, $\gamma$, and $\delta$ polypeptides), which not only contain binding sites for GABA, barbiturates, and benzodiazepines, but also form the chloride channel.\textsuperscript{52,33} Ro15-1788 did not affect the propofol-response (fig. 5A), thereby indicating a possibility that the site of propofol action is different from that of benzodiazepines. This idea was further supported by the results that the propofol response was augmented by diazepam, which does not activate the GABA$_A$ channel by itself (fig. 5B). This notion is in agreement with the results reported in a binding study that propofol
did not displace $^3$H-flunitrazepam from cerebral synaptosomes. Prince and Simmonds examined the temperature dependence of $^3$H-flunitrazepam binding to rat brain membranes in the presence of barbiturates, alphaxalone, and propofol, producing the conclusion that the three anesthetics interact with the GABA$_A$ receptor at distinct recognition sites. Lack of effects of pentobarbital on the propofol response in the current study indicates that the conformation of the receptor bound with propofol is different from that bound with GABA. This notion may provide a basis for the finding of Hales and Lambert that the unit conductances activated by these two agents were not identical.

**Clinical Implications**

The systemic blood concentration of propofol in laboratory animals required to produce unconsciousness was reported to be in the range of 1–4 μg/ml (namely, 5.6–22.4 × 10$^{-6}$ M). The arterial plasma concentration to induce loss of consciousness in humans is reportedly 3–4 μg/ml (16.8–22.4 × 10$^{-6}$ M). This concentration range is consistent with that of the current experiments ($K_0 = 12 × 10^{-6}$ M; fig. 1). A fraction of propofol in blood may, however, bind to plasma proteins, making the concentration of free propofol smaller. It is likely, therefore, that the non-desensitizing chloride current evoked by low concentrations of propofol (fig. 1) plays a significant role in anesthesia in animals and humans. Augmentation of the GABA response by low concentrations of propofol may also be involved, because we have preliminary evidence that a low concentration of the agent significantly enhances the GABA response (unpublished observation).

Continuous application of high concentrations of propofol desensitized the GABA$_A$ receptor (figs. 1A and 4). The desensitization and the suppression of the potassium channel activity (more than 140 × 10$^{-6}$ M; Magnelli et al.) by the drug may make the neurons more excitable. Although the incidence of excitatory effects of propofol seems to be low, some excitatory side effects (tremor, hypertonus, and spontaneous involuntary muscle movements) have been observed during induction of anesthesia with various doses ranging from 1.5 to 3.0 mg/kg body weight. Manifestations of excitation occurred during maintenance in 23% of patients, in which spontaneous movement was most common.

In conclusion, propofol, a hydrophobic intravenous anesthetic, evokes a chloride current in mammalian central neurons at clinically relevant concentrations.

Two or more molecules of propofol cooperatively bind and activate the GABA$_A$ receptor-chloride ionophore complex and increase the chloride conductance, which reduces the excitability of neurons and consequently inhibits polysynaptic excitations in the central nervous system. High concentrations of propofol desensitize the GABA$_A$ receptor, which may result in suppression of the GABA$_A$ inhibitory system.

**References**

31. Magnelli V, Nobile M, Maestrone E: K<sup>＋</sup> channels in PC12 cells are affected by propofol. Pfugers Arch 420:393–398, 1992

Anesthesiology, V 79, No 4, Oct 1993