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Behavior and Cellular Evidence for Propofol-Induced Hypnosis Involving Brain Glycine Receptors

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Abstract

Background—It is well documented that several general anesthetics, including propofol, potentiate glycine receptor function. Furthermore, glycine receptors exist throughout the central nervous system, including areas of the brain thought to be involved in sleep. However, the role of glycine receptors in anesthetic-induced hypnosis has not been determined.

Methods—Experiments were conducted in rats, where the loss of righting reflex (LORR) was used as a marker of the hypnotic state. Propofol-induced LORR was examined in the presence and the absence of strychnine (a glycine receptor antagonist), GABAzine (a γ-aminobutyric acid A receptor antagonist), as well as ketamine (an antagonist of N-methyl-D-aspartic acid subtype of glutamate receptors). Furthermore, the effects of propofol on the currents elicited by glycine and γ-aminobutyric acid were analyzed in neurons isolated from the posterior hypothalamus of rats. The effects of strychnine and GABAzine on propofol-induced currents were also evaluated.

Results—Strychnine and GABAzine dose-dependently reduced the percentage of rats exhibiting LORR induced by propofol. Furthermore, strychnine significantly increased the onset time and reduced the duration of LORR induced by propofol. In contrast, strychnine did not affect the LORR induced by ketamine. Additionally, propofol markedly increased the currents elicited by glycine and GABA of hypothalamic neurons. Conversely, strychnine and GABAzine both profoundly attenuated the current induced by propofol.

Conclusion—Strychnine, the glycine receptor antagonist dose-dependently reduced propofol-induced loss of righting reflex in rats and propofol-induced current of rat hypothalamic neurons. These results suggest that neuronal glycine receptors partially contribute to propofol-induced hypnosis.

Introduction

DETERMINING the relationship between the cellular mechanisms and the behavioral effects of anesthetics is an important objective of anesthesia research. Although propofol is widely used as a sedative-hypnotic agent, the molecular mechanism underlying its action has yet to be completely elucidated. Current evidence indicates that propofol-induced hypnosis is

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Summary Statement: Strychnine, the glycine receptor antagonist dose-dependently reduced propofol-induced loss of righting reflex in rats and propofol-induced current of rat hypothalamic neurons. These results suggest that neuronal glycine receptors partially contribute to propofol-induced hypnosis.

mediated largely by enhancing the function of γ-aminobutyric acid A receptor, in particular those contains the β_3 subunit [1,2,3]. In addition, propofol inhibits hyperpolarization-activated cyclic nucleotide gated channels containing hyperpolarization-activated cyclic nucleotide gated 1 subunits, which is thought to contribute to its inhibition of mouse cortical pyramidal neurons [4].

Like γ-aminobutyric acid, glycine is one of the major inhibitory neurotransmitters in the central nervous system [5,6]. Glycine is thought to predominantly exhibit its effects in the spinal cord and brain stem [7,8]. However, functional glycine receptors (GlyRs) exist throughout the central nervous system, including the hypothalamus [9,10]. Previous studies have demonstrated that propofol potentiated the glycine currents (I_{Gly}) of spinal neurons [11,12], and in an expression system [13]. In particular, propofol has been shown to restore the function of "hyperkplexic" mutant GlyRs [14]. Furthermore, the GlyR antagonist strychnine partially blocked the current elicited by propofol (I_{PRO}) in hypothalamic neurons [15] and in spinal neurons [12]. However, the role of GlyRs in propofol-induced hypnosis has not been determined. In the current study, we performed experiments in rats comparing the loss of righting reflex (LORR) induced by propofol in the absence and presence of strychnine and the γ-aminobutyric acid A receptor antagonist GABAzine. In addition, we evaluated the effects of propofol on the currents induced by glycine (I_{Gly}) and by γ-aminobutyric acid (I_{GABA}), as well as the effects of strychnine and GABAzine on the I_{PRO} in neurons isolated from the posterior hypothalamus, which is a pivotal area in the sleep pathway.

Materials and Methods

Animal preparation

The experimental protocol conformed to the Guide of National Institute of Health for the Care and Use of Laboratory Animals and was fully approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey, Newark, NJ.

For *in vivo* experiments, female adult Sprague-Dawley rats (250-350 g) were individually housed under controlled conditions (20-22°C), with plentiful access to water and food *ad libitum*.

Assessment of hypnosis

The primary endpoint for evaluation of the hypnotic state was the LORR, which was defined as the inability of animals to right themselves when positioned in a supine position. Specifically, after injection of propofol or ketamine, the rats were gently turned onto their backs immediately and thereafter at 5-minute intervals. If they did not regain their posture within 10 seconds, then they were recorded as having lost their righting reflex. The estimation of LORR was made by observers who did not know what prior drug treatment the animals had received. In experiments measuring hypnosis, the righting reflex was considered restored when animals first regained an upright position by standing on their feet. The onset time, duration and percentage of animals exhibiting LORR were measured.

Cannulae implantation

Rats were anesthetized using sodium pentobarbital (20 mg/kg, intraperitoneal injection), and were prepared for aseptic surgery and secured into a stereotaxis frame. Cannulae (22G, 11 mm length) (Plastics One Inc, Roanoke, VA) were positioned for injection into the intracerebralventricular space (ICV) at 5.2 mm anteroposterior, -1.0 mm in the mediolateral, and -9.1 mm dorsoventrical from Bregma [16]. Cannulae were affixed with dental resin (Orthodontic Resin, Caulk Company, Mitford, DE) and animals were allowed to recover for at least seven days. After completion of all experiments, animals were sacrificed by

administering an overdose of sodium pentobarbital and were perfused through the heart with 10% buffered formalin. Frozen sections of the brain were cut on a cryostat (50 μm) and stained with cresyl violet to assess the implantation position of the cannulae.

Isolation of neurons

The brain slices were prepared as described previously [10,17]. In brief, rats, aged 12-24 postnatal days, were anesthetized and then sacrificed by decapitation. The brain was quickly excised and coronally sliced (300 μm) with a VF-200 Slicer (Precisionary Instruments INC, Greenville, NC). This was done in ice-cold modified glycerol-based artificial cerebrospinal fluid saturated with 95%O_2/5% CO_2 (carbogen) containing (in mM): 250 glycerol, 2.5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 26 $NaHCO_3$, and 11 glucose [17]. Midbrain slices were then kept in carbogen-saturated regular artificial cerebrospinal fluid at room temperature (22-24°C) for at least one hour before use. Regular artificial cerebrospinal fluid has the same composition as glycerol-based artificial cerebrospinal fluid except that glycerol was replaced with 126 mM NaCl.

For cell isolation, midbrain slices containing the posterior hypothalamus were first incubated in oxygenated standard extracellular solution containing 0.3 mg/ml papain (from papaya latex; Sigma, St. Louis, MO) at room temperature for 15 minutes. The standard extracellular solution contained (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (320 mOsm, pH set to 7.3 with Tris base). The slices were then incubated in enzyme-free standard extracellular solution. The posterior hypothalamic region was dissected out under an inverted microscope and single cells were dissociated by trituration using two fire-polished glass pipettes with gradually narrowing diameters. The cells settled to the bottom of the culture dish within 20 min and were ready for electrophysiological recordings.

Electrophysiological measurements

Whole-cell currents were recorded at a holding potential of 0 mV using an Axopatch 200B amplifier, via a Digidata 1322A analog-to-digital converter, and pClamp 9.2 software (Molecular Devices Co., Foster city, CA) at room temperature (22- 24 °C), using a pipette solution containing (in mM): 135 CsF, 5 KCl, 2 $MgCl_2$, 10 HEPES, 2 Mg ATP, 0.2 GTP, pH 7.2, osmolarity 280 - 300 mOsm.

Chemicals and application

Propofol, ketamine, glycine, strychnine, and GABAzine (SR-95531) (Sigma Chemical Company, St. Louis, MO) were prepared in normal saline solutions. Solutions were applied to a dissociated neuron with a superfusion system via a Y-tube perfusion system [18]. The propofol used for intravenous administration (IV) was from AstraZeneca Pharmaceuticals LP (Wilmington, DE).

Statistical analysis

Data were statistically compared using a Student's t-test at a significance level of $P < 0.05$ using Sigma plot (Systat Software Inc., San Jose, CA) for statistical analyses. For all experiments, average values are expressed as mean ± SEM. Dose-response data were fitted as previously described [3,19] to a logistic equation of the following form:

$$P=100D^n/(D^n+(ED_{50})^n)$$

where P is the percent of the population anesthetized, D is the drug dose, n is the slope parameter, and ED_{50} is the drug dose for a half-maximal effect.

Results

Strychnine (IP) Attenuates Hypnosis Induced by Propofol (IP), but not by Ketamine (IP)

We first tested whether systemic (IP) administration of strychnine could attenuate propofol-induced hypnosis. We used the LORR score as our primary measure for hypnosis because the concentrations of anesthetics that are necessary to produce the hypnotic state in humans are similar to those needed to induce LORR in rodents [1,3,20,21]. As expected, strychnine (IP) dose-dependently reduced the percentage of rats exhibiting LORR in response to propofol (IP). Specifically, 0.1, 0.3, 0.5, and 0.75 mg/kg strychnine reduced the LORR induced by 100 mg/kg propofol to 100%, 100%, 75%, and 56%, respectively. A comparable result was observed when strychnine was given subcutaneously. Strychnine (0.75 mg/kg, IP) induced a large rightward shift of the LORR dose-response curve to propofol and significantly increased the median effective dose (ED_{50}) of propofol from 69.0 ± 0.8 mg/kg (mean ± SEM) to 96.0 ± 2.3 mg/kg (P = 0.048, Fig. 1A). The subcutaneous administration of GABAzine also reduced the percentage of rats exhibiting LORR in response to propofol (IP). The effect was dependent on the concentrations of GABAzine: 3 and 5 mg/kg GABAzine reduced the LORR induced by 100 mg/kg propofol to 66.6%, and 33.3%, respectively. The systemic administration of GABAzine (5 mg/kg, subcutaneous injection) resulted in a rightward shift of the LORR dose-response curve to propofol and increased the ED_{50} of propofol to 104.5 ± 5.1 mg/kg (P = 0.028, Fig. 1A). There was no significant difference between the dose-response curve to propofol generated in the presence of GABAzine (5 mg/kg, subcutaneous injection) and that in the presence of strychnine (0.75 mg/kg, IP) (P = 0.39). Ketamine exemplifies another class of anesthetics which has no effect on GlyRs, but reduces excitatory neurotransmission by inhibiting the N-methyl-D-aspartic acid subtype of glutamate receptors [22]. The percentage of animals exhibiting LORR in response to 35, 50, and 150 mg/kg (IP) of ketamine was not changed in the absence or the presence of strychnine (0.75 mg/kg, IP, Fig. 1B). Accordingly, the ED_{50} of ketamine was virtually the same in the absence (33.3 ± 0.5 mg/kg) and the presence (33.2 ± 10.4 mg/kg) of strychnine (P > 0.8, Fig. 1B). This finding shows that systemic strychnine does not act `non-specifically' to attenuate responses to all anesthetic agents; for example, by causing a generalized increase in neuronal excitability. These data indicate that propofol is less effective as a hypnotic agent in the presence of a GlyR antagonist and probably exerts its hypnotic effect, in part, through GlyRs. Of note, strychnine reduced LORR in 25% of the (2/8) rats receiving 100 mg/kg ketamine. The underlying mechanism for this effect warrants further investigation.

Strychnine (ICV) Attenuates Hypnosis Induced by Propofol (IV), but not by Ketamine (IV)

To confirm that GlyRs located in the brain are responsible for the effect of strychnine, strychnine was injected into the intracerebralventricular space and propofol (IV) was used to induce LORR in a dose-dependent manner (Fig. 2A1). Strychnine (50 μg/25 μL, ICV) induced a rightward shift in the percentage of rats exhibiting LORR induced by propofol (IP) and increased the median effective dose (ED_{50}) of propofol from 5.18 ± 0.02 mg/kg to 5.53 ± 0.07 mg/kg (P = 0.017, Fig. 2A1).

Additionally, strychnine (50 μg/25 μL, ICV) significantly prolonged the onset time (control, 4.6 ± 0.2 sec; strychnine, 6.0 ± 0.3 sec; P < 0.01, n = 6, Fig. 2A2), and reduced the duration (control, 19.03 ± 3.04 min; strychnine, 10.77 ± 1.39 min; P < 0.05, n = 6) of LORR induced by 10 mg/kg propofol (IV, Fig. 2A3). Similar results were observed when other doses (9 and 12 mg/kg) of propofol were used (Fig. 2A2, A3). Thus, propofol (IV) is less effective as a hypnotic in the presence of strychnine (ICV).

As a comparison, we tested the LORR induced by ketamine (IV) in the absence and presence of strychnine (50 μg/25 μL, ICV). As expected, ketamine (20-30 mg/kg, IV) induced LORR

in a dose-dependent manner. The percent LORR, the onset time, and the duration of LORR induced by ketamine (IV) were essentially the same with or without strychnine (Fig. 2B1-3).

These data indicate that the attenuation of propofol-induced hypnosis by strychnine is a result of blocking the brain GlyRs, instead of non-specific excitatory effects in the central nervous system.

Propofol Enhances I_{Gly} of Hypothalamus Neurons

The above *in vivo* experiments indicate that propofol-induced hypnosis involves brain GlyRs. We next examined the effects of propofol on I_{Gly} using patch clamp techniques. The I_{Gly} were elicited by the application of glycine to neurons isolated from the posterior hypothalamus of rat brains. All neurons tested responded to the application of glycine. The posterior hypothalamus is a key region in the brain sleep pathway [233]. At a holding potential of 0 mV, application of glycine (30 μM) elicited an outward current, which was completely abolished by strychnine (Fig. 3A), indicating the presence of strychnine-sensitive GlyRs in the posterior hypothalamic neurons. When applied together with propofol, the current was substantially larger. However, strychnine eliminated this current, indicating that it was mediated by strychnine-sensitive GlyRs. We next measured the I_{Gly} elicited by 10 μM glycine in the presence of varying concentrations of propofol. When applied separately, glycine and propofol each elicited an outward current (Fig. 3B). To quantify the propofol-glycine interaction, we first normalized the peak current amplitude elicited by 10 μM propofol, or by the mixture of glycine plus propofol to the peak current induced by 10 μM glycine. On average, the peak current amplitude elicited by 10 μM propofol was 108.5 ± 7.5% (n = 7) of that by 10 μM glycine. The peak amplitude elicited by the mixture (10 μM glycine and 10 μM propofol) was 382.2 ± 98.5% (n = 7) of that elicited by 10 μM glycine. This value is considerably greater than the sum of 10 μM glycine and 10 μM propofol (100 + 109 = 209). This result indicates that propofol and glycine have a synergistic effect. Figure 3C summarizes the result of 3 to 7 cells indicating that the peak amplitude of the I_{Gly} increased with the increasing concentrations of propofol. That is, the normalized values of peak amplitude elicited by 10 μM glycine in the presence of 0, 3, 10, 30, and 100 μM propofol were 0, 54.8 ± 5.2% (n = 3, p = 0.06), 382.2 ± 98.5% (n = 7, p <0.01), 385.6 ± 128.9% (n = 5, p < 0.05), and 431.3 ± 29.2% (n = 3, p < 0.01), respectively. The apparent EC_{50} for propofol was 5.4 ± 0.6 μM.

To further characterize the propofol potentiation of I_{Gly}, we evaluated the effects of propofol on currents elicited by varying concentrations of glycine (3, 10, 15, 30, and 100 μM). While propofol profoundly increased the current elicited by 3, 10, and 15 μM glycine, it had no significant effect on the current elicited by 30 and 100 μM glycine (Fig. 4A1-A3, B1, B2). Propofol (10 μM) shifted the concentration-response curve of glycine to the right and decreased the EC_{50} for glycine from 35.7 ± 3.1 μM in controls to 11.1 ± 2.9 μM in the propofol group (Fig. 4A3). Similarly, 1 μM propofol enhanced the currents induced by 10, 15, 30, and 100 μM glycine by 1395 ± 567 % (n = 4, P = 0.03), 741 ± 144% (n = 5, P = 0.01), 17 ± 19% (n = 5, P = 0.5), and 8 ± 15% (n = 5, P = 0.6), respectively. These data suggest that propofol acts on the GlyR to increase its affinity for agonist or it functions as a positive allosteric modulator of GlyRs [12]. Similarly, propofol (1 μM) enhanced currents induced by 0.1, 1, 10, and 100 μM γ-aminobutyric acid by 293 ± 158 % (n = 3, P = 0.2), 497 ± 114% (n = 3, P = 0.04), 55 ± 42% (n = 4, P = 0.16), and -44 ± 16% (n = 3, P = 0.14), respectively (Fig. 4C1, C2).

Propofol alone dose-dependently induced an outward current in hypothalamic neurons (Fig. 5A1). Propofol (1, 3, 10, 30, and 100 μM) induced currents with the amplitudes of 15 ± 5 (n = 5), 35 ± 18 (n = 5), 174 ± 49 (n = 11), 441 ± 85 (n = 11), and 877 ± 199 pA (n = 4), respectively. The apparent EC_{50} of propofol alone was 26.4 ± 3.4 μM (Fig. 5A2). Strychnine (1 μM) and bicuculline (10 μM) suppressed the current induced by 30 μM propofol by 46.6 ± 3.7% (n = 11, P < 0.01) and by 58.9 ± 4.9% (n = 4, P < 0.05), respectively (Fig. 5B1-B3).

Discussion

Our major finding is that the GlyRs in the brain contribute to propofol-induced hypnosis. Our result shows that strychnine reduced the percentage of animals exhibiting LORR in response to propofol, prolonged the onset time but reduced the duration of LORR induced by propofol, and did not alter the LORR induced by ketamine. These results indicate that strychnine attenuation of propofol-induced LORR is a result of the blockade of brain GlyRs, instead of a generalized increase in neuronal excitability. Finally, in keeping with *in vivo* observations, our *in vitro* patch-clamp data indicate that propofol potentiates the I_{Gly} from posterior hypothalamic neurons. Thus, the current investigation provides the first behavioral and cellular evidence indicating that brain GlyRs contribute to the hypnotic effect of propofol.

Interestingly, systematic strychnine produced an effect which is comparable to that of systemic GABAzine regarding the attenuation of LORR induced by propofol (Fig. 1A). These data indicate that both GlyRs and γ-aminobutyric acid A receptors play a crucial role in the propofol-induced hypnotic state. Our data are in general agreement with a previous study showing that γ-aminobutyric acid A receptor blockade antagonizes the action of propofol but not ketamine [3,24]. Based on our observations, we hypothesize that propofol potentiates the function of GlyRs in neurons within the sleep pathway, increases the influx of chloride ions, and hyperpolarizes neurons. Thus, these appear to be, at least in part, the cellular and molecular consequences of propofol administration that contribute to its hypnotic effect.

Moreover, our *in vivo* observations are supported by the results of our *in vitro* experiments. Our patch clamp data indicate that functional GlyRs exist in posterior hypothalamic neurons. Propofol significantly enhanced I_{Gly}, indicating that these GlyRs are sensitive to propofol. Our *in vitro* observation is consistent with previous studies regarding propofol potentiation of I_{Gly} [12,13]. In addition, we observed that propofol substantially enhanced I_{GABA}, which is consistent with previous reports [11-13,15,25]. Interestingly, the I_{PRO} of neurons from different brain areas appear to have significantly different sensitivities to strychnine. Strychnine (1 μM) produced a 47% inhibition of the I_{PRO} of posterior hypothalamic neurons, but only a 10% inhibition of the I_{PRO} of hypothalamic paraventricular nucleus neurons [15]. We interpret these data to signify differences among brain regions.

GlyRs are modulated by a number of drugs, including volatile and intravenous anesthetics [26]. Volatile anesthetics, such as halothane, chloroform, and ether enhance the function of GlyRs in rat medullary neurons [27], in rat hippocampal neurons [28], in recombinant systems with transiently transfected cells [29], and in Xenopus oocytes [27,30]. GlyRs in the spinal cord have been recognized as the most credible candidates for mediating immobility caused by volatile anesthetics [31,32]. Furthermore, some intravenous anesthetics such as propofol and pentobarbital potentiate the cellular response to glycine in a homomeric expression system [30] and in spinal dorsal horn neurons [12]. Our current investigation provides compelling evidence that brain GlyRs play a considerable role in propofol-induced hypnosis.

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References

1. Rudolph U, Antkowiak B. Molecular and neuronal substrates for general anaesthetics. Nat Rev Neurosci 2004;5:709–20. [PubMed: 15322529]
2. Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. FASEB J 2003;17:250–2. [PubMed: 12475885]
3. Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. Nat Neurosci 2002;5:979–84. [PubMed: 12195434]
4. Chen X, Shu S, Bayliss DA. Suppression of ih contributes to propofol-induced inhibition of mouse cortical pyramidal neurons. J Neurophysiol 2005;94:3872–83. [PubMed: 16093340]
5. Betz H. Structure and function of inhibitory glycine receptors. Q Rev Biophys 1992;25:381–94. [PubMed: 1284091]
6. Kuhse J, Betz H, Kirsch J. The inhibitory glycine receptor: architecture, synaptic localization and molecular pathology of a postsynaptic ion-channel complex. Curr Opin Neurobiol 1995;5:318–23. [PubMed: 7580154]
7. Curtis DR, Hosli L, Johnston GA. A pharmacological study of the depression of spinal neurones by glycine and related amino acids. Exp Brain Res 1968;6:1–18. [PubMed: 5721755]
8. Young AB, Snyder SH. Strychnine binding associated with glycine receptors of the central nervous system. Proc Natl Acad Sci U S A 1973;70:2832–6. [PubMed: 4200724]
9. Akaike N, Kaneda M. Glycine-gated chloride current in acutely isolated rat hypothalamic neurons. J Neurophysiol 1989;62:1400–9. [PubMed: 2600631]
10. Ye JH, McArdle JJ. 2,3-Butanedione monoxime modifies the glycine-gated chloride current of acutely isolated murine hypothalamic neurons. Brain Res 1996;735:20–9. [PubMed: 8905165]
11. Hales TG, Lambert JJ. The actions of propofol on inhibitory amino acid receptors of bovine adrenomedullary chromaffin cells and rodent central neurones. Br J Pharmacol 1991;104:619–28. [PubMed: 1665745]
12. Dong XP, Xu TL. The actions of propofol on gamma-aminobutyric acid-A and glycine receptors in acutely dissociated spinal dorsal horn neurons of the rat. Anesth Analg 2002;95:907–14. [PubMed: 12351266]
13. Pistis M, Belelli D, Peters JA, Lambert JJ. The interaction of general anaesthetics with recombinant GABAA and glycine receptors expressed in Xenopus laevis oocytes: a comparative study. Br J Pharmacol 1997;122:1707–19. [PubMed: 9422818]
14. O'Shea SM, Becker L, Weiher H, Betz H, Laube B. Propofol restores the function of “hyperekplexic” mutant glycine receptors in Xenopus oocytes and mice. J Neurosci 2004;24:2322–7. [PubMed: 14999083]
15. Shirasaka T, Yoshimura Y, Qiu DL, Takasaki M. The effects of propofol on hypothalamic paraventricular nucleus neurons in the rat. Anesth Analg 2004;98:1017–23. [PubMed: 15041591]
16. Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates - The New Coronal Set. Vol. Sixth Edition. Academic press; Elsevier Inc.: 2007.
17. Ye JH, Zhang J, Xiao C, Kong JQ. Patch-clamp studies in the CNS illustrate a simple new method for obtaining viable neurons in rat brain slices: glycerol replacement of NaCl protects CNS neurons. J Neurosci Methods 2006;158:251–9. [PubMed: 16842860]
18. Zhou C, Xiao C, McArdle JJ, Ye JH. Mefloquine enhances nigral gamma-aminobutyric acid release via inhibition of cholinesterase. J Pharmacol Exp Ther 2006;317:1155–60. [PubMed: 16501066]
19. Waud DR. On biological assays involving quantal responses. J Pharmacol Exp Ther 1972;183:577–607. [PubMed: 4636393]
20. Bonin RP, Orser BA. GABA(A) receptor subtypes underlying general anesthesia. Pharmacol Biochem Behav 2008;90:105–12. [PubMed: 18201756]
21. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 2008;9:370–86. [PubMed: 18425091]

22. Anis NA, Berry SC, Burton NR, Lodge D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. Br J Pharmacol 1983;79:565–75. [PubMed: 6317114]

23. Nitz D, Siegel JM. GABA release in posterior hypothalamus across sleep-wake cycle. Am J Physiol 1996;271:R1707–12. [PubMed: 8997373]

24. Sonner JM, Zhang Y, Stabernack C, Abaigar W, Xing Y, Laster MJ. GABA(A) receptor blockade antagonizes the immobilizing action of propofol but not ketamine or isoflurane in a dose-related manner. Anesth Analg 2003;96:706–12. [PubMed: 12598250]

25. Patten D, Foxon GR, Martin KF, Halliwell RF. An electrophysiological study of the effects of propofol on native neuronal ligand-gated ion channels. Clin Exp Pharmacol Physiol 2001;28:451–8. [PubMed: 11380521]

26. Yamakura T, Bertaccini E, Trudell JR, Harris RA. Anesthetics and ion channels: molecular models and sites of action. Annu Rev Pharmacol Toxicol 2001;41:23–51. [PubMed: 11264449]

27. Downie DL, Hall AC, Lieb WR, Franks NP. Effects of inhalational general anaesthetics on native glycine receptors in rat medullary neurones and recombinant glycine receptors in Xenopus oocytes. Br J Pharmacol 1996;118:493–502. [PubMed: 8762070]

28. Kira T, Harata N, Sakata T, Akaike N. Kinetics of sevoflurane action on GABA- and glycine-induced currents in acutely dissociated rat hippocampal neurons. Neuroscience 1998;85:383–94. [PubMed: 9622238]

29. Harrison NL, Kugler JL, Jones MV, Greenblatt EP, Pritchett DB. Positive modulation of human gamma-aminobutyric acid type A and glycine receptors by the inhalation anesthetic isoflurane. Mol Pharmacol 1993;44:628–32. [PubMed: 7690453]

30. Daniels S, Roberts RJ. Post-synaptic inhibitory mechanisms of anaesthesia; glycine receptors. Toxicol Lett 1998;100-101:71–6. [PubMed: 10049183]

31. Sonner JM, Antognini JF, Dutton RC, Flood P, Gray AT, Harris RA, Homanics GE, Kendig J, Orser B, Raines DE, Rampil IJ, Trudell J, Vissel B, Eger EI 2nd. Inhaled anesthetics and immobility: mechanisms, mysteries, and minimum alveolar anesthetic concentration. Anesth Analg 2003;97:718–40. [PubMed: 12933393]

32. Zhang Y, Laster MJ, Hara K, Harris RA, Eger EI 2nd, Stabernack CR, Sonner JM. Glycine receptors mediate part of the immobility produced by inhaled anesthetics. Anesth Analg 2003;96:97–101. [PubMed: 12505932]

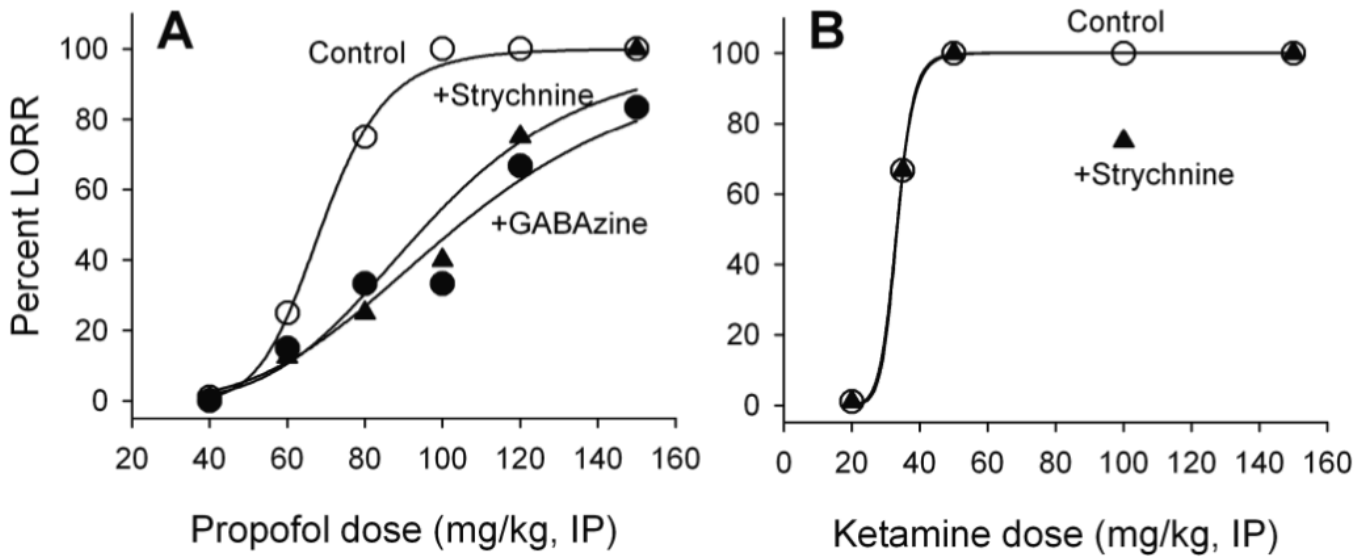

Fig. 1.

Strychnine (▲0.75 mg/kg, intraperitoneal injection, IP) and GABAzine (•5 mg/kg, subcutaneous injection) decrease the percentage of rats exhibiting loss of righting reflex (Percent LORR) induced by propofol (○, IP, A) but not by ketamine (○, IP, B). Minimum cohort size is six. The solid lines are the fit to the data, obtained with the logistic equation described in the method section. The EC50 (± SEM) and Hill coefficient (± SEM) values are 69.0 ± 0.8 mg/kg and 8.2 ± 0.6 for propofol alone, 96.0 ± 2.3 mg/kg and 4.6 ± 1.2 for propofol + strychnine, and 104.5 ± 5.1 mg/kg and 3.8 ± 0.76 for propofol + GABAzine, 33.3 ± 0.5 mg/kg and 13.9 ± 4.3 for ketamine alone, and 33.2 ± 10. 4 mg/kg and 12.9 ± 74.9 for ketamine + strychnine, respectively.

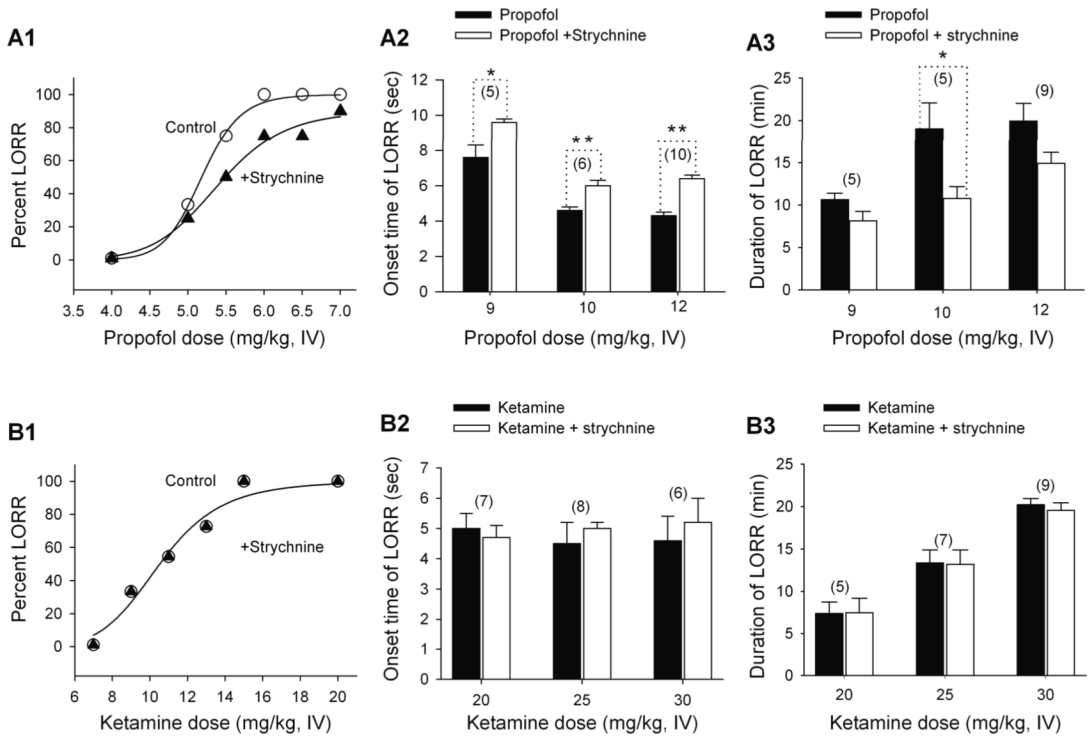

Fig. 2.

Strychnine (50μg/25μL, intracranial ventricular space injection) decreases the percentage of rats exhibiting loss of righting reflex (Percent LORR, A1), increases the onset time (A2), and reduces the duration (A3) of LORR induced by propofol (intravenous injection, IV), but not by ketamine (intravenous injection, B1-3). Numbers of rats in each group is indicated. *, $P < 0.05$, **, $P < 0.01$, propofol verses propofol + strychnine. The solid lines are the fit to the data, obtained with the logistic equation described in the method section. The EC50 (± SEM) and Hill coefficient (± SEM) values are 5.18 ± 0.02 mg/kg and 20.6 ± 1.9 for propofol alone, 5.53 ± 0.07 mg/kg and 9.4 ± 1.3 for propofol + strychnine, 10.5 ± 0.3 mg/kg and 6.4 ± 1.2 for ketamine alone and ketamine + strychnine, respectively.

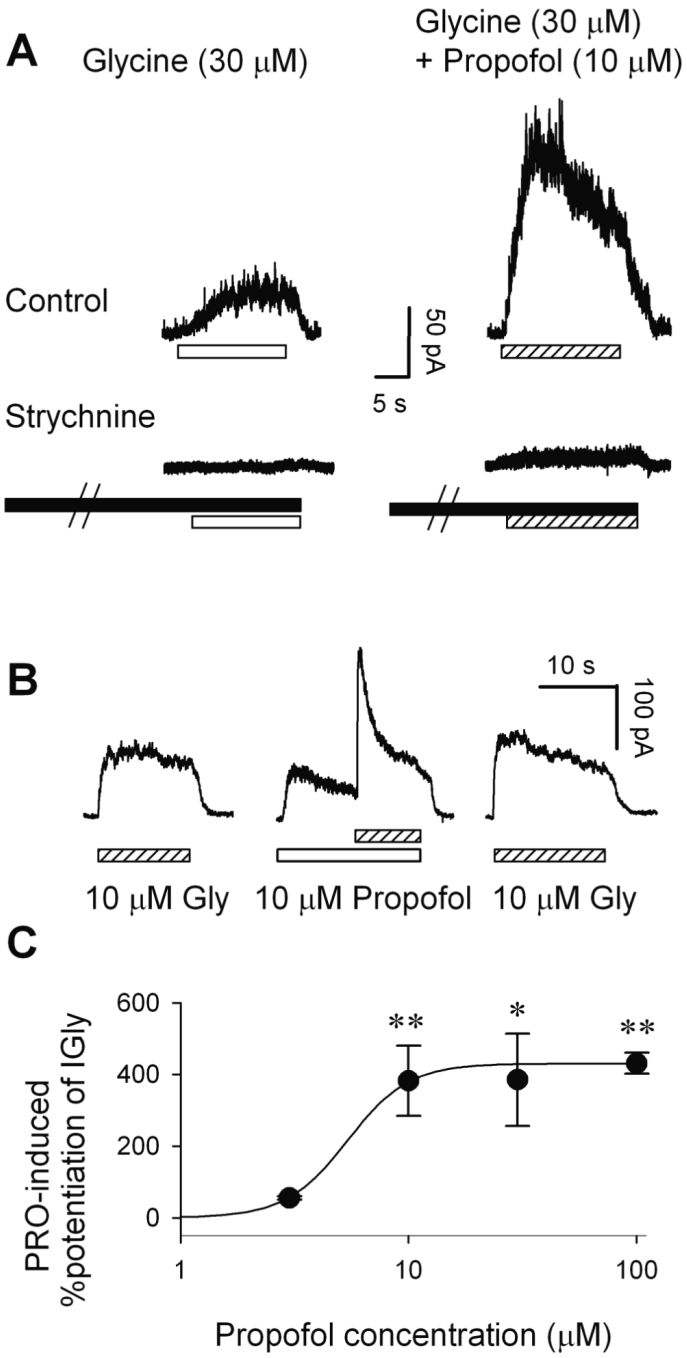

Fig. 3.

Strychnine abolishes glycine currents of rat posterior hypothalamic neurons in the absence and presence of propofol (A). B, Current traces elicited by glycine (Gly, 10 μM) and propofol (10 μM), applied separately and in combination (as indicated). C, The dose-response relationship for propofol (PRO)-induced potentiation of currents elicited by 10 μM glycine. Each data point is the mean ± s.e.m. of 3 to 7 neurons. * $P < 0.05$, ** $P < 0.01$, propofol + 10 μM glycine verses 10 μM glycine alone. The solid line is least square fit of the following form of Michaelis-Menten equation to the experimental data: $I = (IMax * C^n)/(C^n + EC50^n)$, where I, IMax, C, EC50 and *n* are glycine current, maximal glycine current, propofol concentration, the concentration of propofol at which the glycine current is 50% of maximum and the Hill

coefficient, respectively. The EC50 (SEM) and Hill coefficient (SEM) values are 5.4 ± 0.6 μM and 3.2 ± 0.6, respectively.

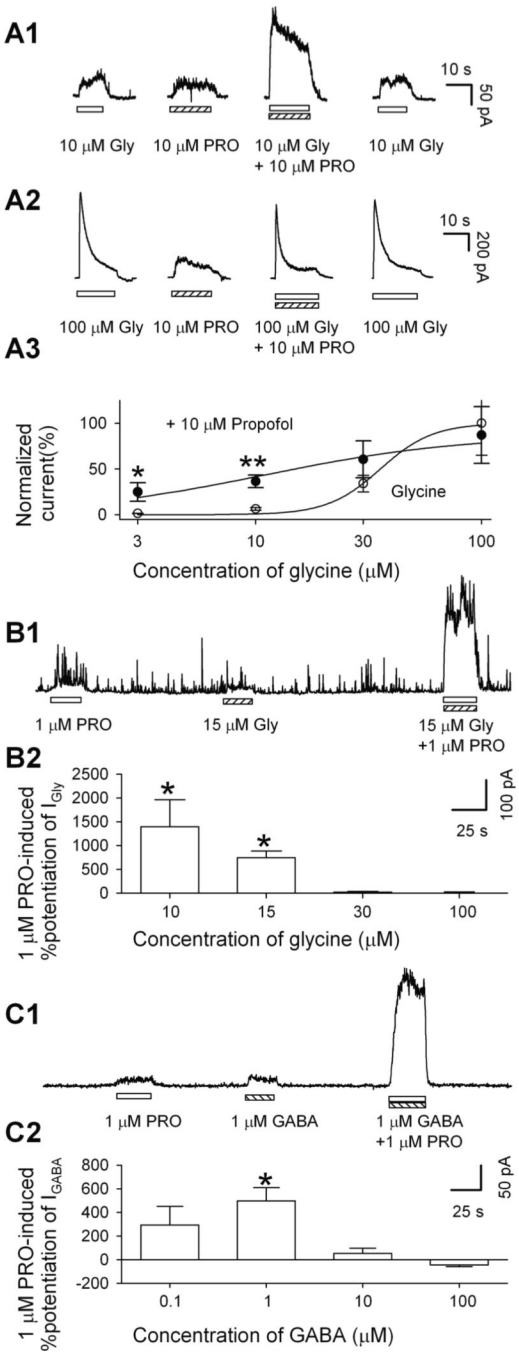

Fig. 4.

Propofol (PRO) enhances currents induced by glycine and by GABA of posterior hypothalamic neurons in response to subsaturating concentrations of the agonist. Propofol (1 and 10 μM) increased the amplitude of glycine currents induced by 3, 10, and 15 μM glycine (A1, A3, B1, and B2), but not by 30 and 100 μM glycine (A2, A3, and B2). Each data point is the mean ± SEM of 4 to 5 neurons. The solid lines are least square fit of the Michaelis-Menten equation described in Fig. 3 to the experimental data. The EC50 and Hill coefficient values are 35.7 ± 3.1 μM and 3.7 ± 1.6 for glycine alone, and 11.1 ± 2.9 μM, 1.0 ± 0.3 for glycine + 10 μM propofol, respectively. Note that the trace in B1 was recorded from a cell that was isolated using an enzyme free procedure. This neuron preserved some γ-aminobutyric acid-releasing

terminals 18. Therefore, some spontaneous events were seen. Propofol (1 μM) increased the amplitude of the current induced by 1 μM γ-aminobutyric acid, but not by 10 and 100 μM γ-aminobutyric acid (C1, C2). Each data point is the mean ± SEM of 3 to 5 neurons.

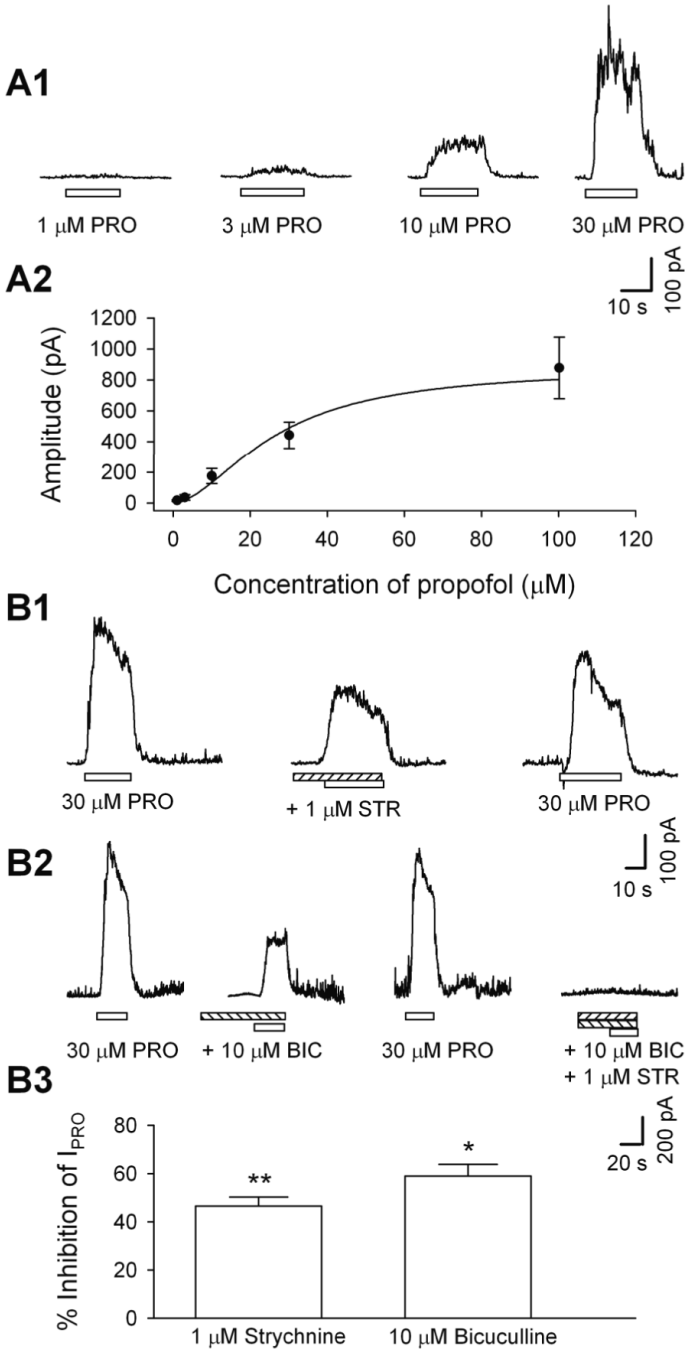

Fig. 5.

Currents induced by propofol (IPRO) from isolated hypothalamic neurons. (A) Representative current traces induced by various concentrations of propofol. A2, Peak amplitude (mean ± SEM of 4 to 12 cells) of the IPRO against the concentrations of propofol. The solid line is least square fit of the Michaelis-Menten equation described in Fig. 3 to the experimental data. The EC50 and Hill coefficient values are 26.4 ± 3.4 μM and 1.8 ± 0.4, respectively. B, Representative traces of propofol-induced current in the presence of strychnine (1 μM) (B1), bicuculline (10 μM), or both (B2). B3, mean ± SEM for strychnine or bicuculline inhibition of propofol-induced current.