Enhancement of \(\gamma\)-Aminobutyric Acid\(_A\) Receptor Activity by \(\alpha\)-Chloralose\(^1\)

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**ABSTRACT**

\(\alpha\)-Chloralose is widely used as an anesthetic in the laboratory due to its minimal effects on autonomic and cardiovascular systems, yet little is known about its mechanism of action. We examined the effects of \(\alpha\)-chloralose on \(\gamma\)-aminobutyric acid type A (GABA\(_A\)) receptor activity because recent studies have shown that several classes of general anesthetics modulate the function of this receptor. GABA\(_A\) receptor activity was assayed by measuring the GABA-induced current in Xenopus oocytes expressed with human GABA\(_A\) receptor alpha-1, beta-1 and gamma-2L subunits. \(\alpha\)-Chloralose produced a concentration-dependent potentiation of the GABA-induced current with an EC\(_{50}\) value of 49 \(\mu\)M and a maximal effect of 239% of control. Membrane current was not affected by \(\alpha\)-chloralose in the absence of GABA. \(\alpha\)-Chloralose (100 \(\mu\)M) increased the affinity for GABA 5-fold and produced a small (17%) increase in the efficacy of GABA. Measurement of the reversal potentials for the \(\alpha\)-chloralose response suggested that the effect is mediated through increased Cl\(^-\) conductance. Studies of \(\alpha\)-chloralose interactions with other allosteric modulators determined that \(\alpha\)-chloralose binds to a site on the GABA\(_A\) receptor complex distinct from the benzodiazepine, neurosteroid and barbiturate sites. Chloral hydrate, trichloroethanol and urethane also augmented GABA-induced currents. \(\alpha\)-Chloralose had no effect on the hydroxytryptamine-induced currents in oocytes expressed with the 5-hydroxytryptamine\(_A\) receptor. These data extend the number of classes of anesthetics that allosterically modulate GABA\(_A\) receptor activity and indicate that GABA\(_A\) receptors may be a common site of action for diverse classes of general anesthetics.

General anesthetics have been in use for >150 years, yet only within the past 10 to 15 years have we begun to understand the molecular mechanism of action of these compounds. Recent research has focused on examining effects of anesthetics on ion channels in neuronal membranes. To date, each of the anesthetics studied modulate GABA\(_A\) receptor activity by enhancing the GABA-induced chloride conductance (Harris et al., 1995). Previous work has primarily focused on the anesthetics used in the clinic; however, little work has been done with nonvolatile anesthetics used in the laboratory and by veterinarians. \(\alpha\)-Chloralose is frequently used as an anesthetic due to its ease of administration, long duration and maintenance of autonomic reflexes (Balis and Monroe, 1964; Silverman and Muir, 1993), yet despite the wide application of this agent, there is little understanding of the mechanisms of action of this anesthetic. Limited studies suggest that \(\alpha\)-chloralose affects GABA\(_A\) receptor activity (Nicoll and Wogtowicz, 1980; Moody et al., 1988; Ishizuka et al., 1989; Kumamoto and Murata, 1996); however, the possibility of \(\alpha\)-chloralose for modulation of GABA\(_A\) receptor activity, mechanism of action and binding site on the receptor complex is not known. Using GABA\(_A\) receptors expressed in Xenopus laevis oocytes as a model system, the effects of \(\alpha\)-chloralose on GABA-induced currents were examined to determine whether modulation of GABA\(_A\) receptor activity may play a role in the anesthetic action of this compound.

**Methods**

**Materials.** Streptomycin, gentamicin sulfate, theophylline, pyruvate, collagenase, tricaine (3-aminobenzoc acid ethyl ester), pentobarbital, \(\alpha\)-chloralose, \(\beta\)-chloralose, chloral hydrate, urethane and trichloroethanol were purchased from Sigma Chemical (St. Louis, MO). GABA, 3\(\alpha\)-OH-DHP and 3\(\beta\)-OH-DHP were purchased from Research Biochemicals (Natick, MA). Flumazenil was a gift from Hoffmann-La Roche (Nutley, NJ). All other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ). \(\alpha\)-Chloralose, \(\beta\)-chloralose, 3\(\alpha\)-OH-DHP and 3\(\beta\)-OH-DHP were dissolved in DMSO and then diluted to final concentrations in Barth’s solution. In most experiments, the concentration of DMSO in the drug solutions did not exceed 0.1%.

**Oocyte preparation.** X. laevis were purchased from Nasco (Fort Atkinson, WI) and kept at a 12:12-hr light/dark cycle. Frogs were

**ABBREVIATIONS:** GABA, \(\gamma\)-aminobutyric acid; ANOVA, analysis of variance; 3\(\alpha\)-OH-DHP, 3\(\alpha\)-hydroxy-5\(\alpha\)-pregnan-20-one; 3\(\beta\)-OH-DHP, 3\(\beta\)-hydroxy-5\(\alpha\)-pregnan-20-one; DMSO, dimethylsulfoxide; HEPES, N-2-hydroxyethyl-piperazine-N’-2-ethanesulfonic acid; 5-HT, 5-hydroxytryptamine.
anesthetized by immersion in 0.1% tricaine for 30 min. A 5-mm incision was made in the abdominal wall and a piece of the ovary was removed and rinsed in collagenase solution (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 5 mM HEPES, pH 7.6). The follicular layer was removed from the oocytes by incubating the ovary with collagenase (2 mg/ml in collagenase solution) for 90 min at 18°C. The oocytes were maintained at 18°C in modified Barth’s solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 5 mM HEPES, pH 7.6) containing 100 mg/liter streptomycin, 50 mg/liter gentamicin sulfate, 500 μM theophylline and 2.5 mM pyruvic acid. A Nanolab microinjector (Drummond Scientific, Broomall, PA) was used to inject oocytes with 50 nl of either a mixture of human GABA<sub>A</sub> receptor alpha<sub>1</sub>, beta<sub>1</sub>-1, and gamma-2L subunit cRNA (0.2–0.5 ng/ml/subunit cRNA) or mouse 5-HT<sub>3</sub> receptor cRNA (1.8 ng/ml). cRNA was prepared from full-length cDNA clones of the human alpha<sub>1</sub>-1, gamma<sub>1</sub>-1, or beta<sub>1</sub>-1 (pCLL811, Garrett et al., 1988), beta<sub>1</sub>-1 (pCLL611, Garrett et al., 1989) and gamma-2L GABA<sub>A</sub> receptor subunits or the mouse 5-HT<sub>3</sub> receptor cDNA. cRNA clones were linearized with XbaI (alpha<sub>1</sub> and gamma-2L), HindIII (beta<sub>1</sub>-1) or NotI (5-HT<sub>3</sub>), and transcribed with either T3 (alpha<sub>1</sub>, 5-HT<sub>3</sub>) or T7 (beta<sub>1</sub>-1, gamma-2L) RNA polymerase and capped using the mMessage Machine kit (Ambion, Austin, TX). This combination of GABA<sub>A</sub> receptor subunits have been used as a model system to study the interactions of several classes of compounds on GABA<sub>A</sub> receptors (Lan et al., 1990; Malherbe et al., 1990; Shingai et al., 1991; Lin et al., 1993a, Mihic et al., 1997).

Electrophysiology. Electrophysiological studies were performed 48 to 72 hr after injection of oocytes with cRNA. Oocytes were placed in a 250-μl recording chamber (Warner Instruments, Hamden, CT), and drug-induced currents were measured using standard two-electrode voltage clamp techniques with a Dagan TEV-201 amplifier. Electrodes were filled with 3 M KCl and had resistances between 0.5 to 2 MΩ. Data were acquired using the pCLAMP 6.0 computer program with a TL-1 interface (Axon Instruments, Foster City, CA). Oocytes were clamped at −70 mV and perfused with Barth’s solution at 1 ml/min by gravity flow. Oocytes were maintained at room temperature (21–23°C) during the electrophysiological experiments. Drugs were delivered to the oocytes by gravity flow using an Auto-bank 8 perfusion system (AutoMate Scientific, Oakland, CA) at 2.4 ml/min. Base-line currents were recorded for 10 sec followed by a 15-sec drug application. The entire drug response was recorded for 1 sec with 100 μM GABA returned to control current. Differences in the responses were determined using Student’s paired t test or a randomized one-way analysis of variance with repeated measures with Tukey’s range test (GBSTAT, Dynamic Microsystems, Silver Spring, MD). The significance level was set at P < .05.

Results

X. laevis oocytes expressed with human GABA<sub>A</sub> receptor alpha<sub>1</sub>, beta<sub>1</sub>-1 and gamma-2L subunits were perfused with 30 μM GABA for 15 sec to determine the control response. After a 5- to 10-min wash period, the oocytes were perfused for 10 sec with 100 μM α-chloralose followed by perfusion for 15 sec with 100 μM α-chloralose plus 30 μM GABA (fig. 1). Perfusion of the oocytes with 100 μM α-chloralose had no direct effect on membrane conductance in the absence of GABA but produced a 2-fold potentiation in the GABA-induced currents (fig. 1). Preapplication of α-chloralose was not required for the response; therefore, in the following experiments, GABA and drugs were mixed and applied together. The level of expression of GABA<sub>A</sub> receptors varied among oocytes and across donor frogs. The current produced by 30 μM GABA ranged from 18 to 184 nA with a mean and S.E. of 58 ± 5 (n = 46). However, the degree of enhancement of the GABA-induced current produced by α-chloralose was quite consistent when expressed as percentage of control current produced by 30 μM GABA alone. The effects of α-chloralose were reversible. Responses to 30 μM GABA returned to control within 5 min of perfusion with α-chloralose.

The effects of α-chloralose were studied further by comparing its concentration-dependent responses with those of pentobarbital, a well-characterized allosteric modulator of GABA<sub>A</sub> receptors. Both α-chloralose and pentobarbital produced inverted U-shape concentration-response curves. α-Chloralose significantly (P < .05, one-way ANOVA) enhanced GABA-induced currents from 32 μM to 10 mM and plateaued at 316 μM (fig. 2). Current responses oscillated and were very erratic at 10 mM for both α-chloralose and pentobarbital and were significantly less (P < .01, one-way ANOVA) than their maximal responses at 316 μM. The EC<sub>50</sub> value of α-chloralose was significantly less (P < .05, Student’s t test) than that of pentobarbital; however, the maxi-

![Fig. 1. Representative digitized recordings of inward currents induced by 30 μM GABA in the absence and presence of 100 μM α-chloralose.](image-url)
of GABA, which was reflected by a 5-fold decrease in the EC50
The Emax values for DMSO, the solvent for no effect on membrane conductance in the absence of GABA.
4 to 7 oocytes from two or three donor frogs.

Fig. 2. Comparison of the concentration dependent effects of α-chloralose and pentobarbital on GABA-induced currents. Oocytes were perfused with 30 μM GABA in the presence of varying concentrations of either α-chloralose or pentobarbital. The EC50 values for α-chloralose and pentobarbital are 49 ± 7 μM (n = 7) and 95 ± 12 μM (n = 4), respectively. The Emax values for α-chloralose and pentobarbital are 239 ± 18 (n = 7) and 566 ± 51 (n = 4), respectively. Values represent the mean ± S.E. for 4 to 7 oocytes from two or three donor frogs.

Fig. 3. Effects of α-chloralose on GABA concentration-dependent responses. Oocytes were perfused with varying concentrations of GABA in the presence and absence of 100 μM α-chloralose. The curves are the mean ± S.E. of values of concentration-response curves determined from 6 oocytes from three donor frogs. For each oocyte, a GABA concentration-response curve was performed in the presence and absence of α-chloralose. For each concentration-response curve, the data were normalized to the maximal response of the GABA concentration-response curves in the absence of α-chloralose. The EC50 values for GABA in the absence and presence of 100 μM α-chloralose was 42 ± 5.1 and 8 ± 0.5, respectively.

Fig. 4. Current-voltage (I-V) relationship of the GABA and α-chloralose responses. Oocytes were clamped at −120 mV and perfused with either 30 μM GABA or 30 μM GABA plus 100 μM α-chloralose. At the peak of the response, a continuous voltage ramp from −120 to +70 mV was applied over a 750-msec period. An I-V curve was recorded before the application of drugs to determine the baseline response. The base-line current was digitally subtracted from the drug-induced response, and the resulting data were plotted as a function of membrane potential. The I-V curves for the GABA and GABA plus chloralose response were measured in each oocyte in normal Barth’s solution (Cl− = 103 mM) and in Barth’s solution with 30 mM Cl−.

significant (P < .05, Student’s t test) increase in the maximal current.
Reversal potentials of the currents were characterized to determine the ion selectivity of the α-chloralose response. The reversal potential for the α-chloralose response was identical to the GABA current in the absence of α-chloralose (fig. 4, table 1). Reversal potentials were measured in Barth’s solution with reduced Cl− concentration to determine if the drug-induced effects were mediated by chloride conductance. Lowering the Cl− concentration to 30 mM produced a significant shift in the reversal potentials for GABA and GABA plus α-chloralose responses to more positive voltages (fig. 4, table 1). It is important to note that there were no differences between the reversal potentials of the GABA and GABA plus α-chloralose responses in 30 mM Cl− buffer. The current-voltage curve was made more linear by α-chloralose, resulting in a greater potentiation of the GABA response at more hyperpolarizing membrane potentials in both normal and 30 mM Cl− buffer (fig. 4).

The GABAA receptor complex contains numerous binding sites for allosteric modulation of the receptor. Three experiments were performed to determine whether α-chloralose modulates GABAA receptor activity by binding to well-characterized allosteric sites on the complex. The first experiment examined the potential interaction of α-chloralose with the benzodiazepine binding site. Enhancement of GABA-induced current by α-chloralose was not affected by the benzodiazepine antagonist flumazenil (data not shown). GABAA receptors also contains binding sites for neurosteroids, but unfortunately, there are no antagonists for this site. 3β-OH-DHP, however, is a weak partial agonist and has been used to antagonize the effects of neurosteroid full agonists (Prince and Simmonds, 1993). In the second experiment, 3β-OH-DHP (10 μM, the highest soluble concentration) produced a small increase in GABA-induced currents that was not statistically significant. Preliminary experiments showed that 3α-OH-DHP, a neurosteroid full agonist, produced a concentration-dependent enhancement in GABA-induced currents that plateaued at 1 μM (data not shown). 3β-OH-DHP (10 μM) inhibited the response produced by 1 μM 3α-OH-DHP by
49% but had no effect on the response elicited by 100 μM \(a\)-chloralose (fig. 5A).

The third experiment examined the potential interaction of \(a\)-chloralose with the barbiturate binding site on the complex. Unfortunately, antagonists for the barbiturate site are not available, so an indirect approach was used to examine the potential binding of \(a\)-chloralose to this site. Figure 2 showed that the efficacy of \(a\)-chloralose was approximately half that of pentobarbital. If \(a\)-chloralose binds to the barbiturate site, it would be acting as a partial agonist and could compete with pentobarbital for the site. Perfusion of the oocytes with GABA in the presence of the EC100 concentrations of both pentobarbital and \(a\)-chloralose should result in a response less than the maximal barbiturate response. The interaction of \(a\)-chloralose with the barbiturate site was investigated by measuring GABA-induced current in the presence of 300 μM pentobarbital, 300 μM \(a\)-chloralose or 300 μM pentobarbital plus 300 μM \(a\)-chloralose. Enhancement of GABA-induced current by 300 μM \(a\)-chloralose was 39% of the 300 μM pentobarbital response. \(a\)-Chloralose did not affect pentobarbital potentiation of GABA-induced currents because potentiation of the GABA-induced response by \(a\)-chloralose plus pentobarbital was similar to the potentiation by pentobarbital alone and significantly greater than the response to \(a\)-chloralose (fig. 5B).

Several other general anesthetics were tested for their ability to affect GABA responses (fig. 6). Chloral hydrate produced a concentration-dependent enhancement of the GABA response. Significant enhancement was observed at concentrations >1 mM. Trichloroethanol, an active metabolite of both \(a\)-chloralose and chloral hydrate (Silverman and Muir, 1993), produced a concentration-dependent augmentation of GABA-induced currents that plateaued at 10 mM.

### Table 1

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<thead>
<tr>
<th>Extracellular chloride concentration</th>
<th>103 mM</th>
<th>30 mM</th>
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<tbody>
<tr>
<td>GABA</td>
<td>-13 ± 6.1</td>
<td>+12 ± 5.7*</td>
</tr>
<tr>
<td>GABA + (a)-chloralose</td>
<td>-13 ± 5.4</td>
<td>+13 ± 3.1*</td>
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* Statistical differences are observed in the reversal potentials between the 103 mM Cl\(^-\) and 30 mM Cl\(^-\) solutions for each drug response (P < .01).

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Fig. 5. Interaction of \(a\)-chloralose with neurosteroid and barbiturate binding sites on the GABA\(_A\) receptor complex. A, Effects of 3\(\beta\)-OH-DHP on \(a\)-chloralose potentiation of GABA-induced current. Oocytes were perfused with 30 μM GABA in the presence of 30 μM 3\(\beta\)-OH-DHP, 1 μM 3\(\alpha\)-OH-DHP, 30 μM 3\(\alpha\)-OH-DHP plus 30 μM 3\(\beta\)-OH-DHP, 100 μM \(a\)-chloralose or 100 μM \(a\)-chloralose plus 30 μM 3\(\beta\)-OH-DHP. Values represent the mean ± S.E. for 6–9 oocytes from four donor frogs. * P < .05 vs. 30 μM 3\(\beta\)-OH-DHP response (paired Student’s t test). ** P < .05 vs. 30 μM \(a\)-chloralose + 30 μM pentobarbital response (one-way ANOVA).
Urethane potentiated GABA-induced currents at concentrations ≥30 mM. β-Chloralose, an inactive isomer of α-chloralose, also was tested. At 10 μM (the limit of its aqueous solubility), β-chloralose had no effect on GABA-induced current or on the α-chloralose (100 μM) potentiation of the GABA response (data not shown).

The pharmacological selectivity of α-chloralose was examined by determining its effects on another member of the family of ligand-gated channels, the 5-HT3 receptor. α-Chloralose, at concentrations up to 1 mM, had no effect on the serotonin-induced currents in X. laevis oocytes expressed with the murine 5-HT3 receptor (data not shown).

**Discussion**

Recently, there has been a reevaluation of the mechanism of action of anesthetics. Theories have shifted from generalized effects on the lipids in the plasma membrane to more selective effects on ion channels, in particular, ligand-gated channels (Franks and Lieb, 1994; Harris et al., 1995). Most clinically used volatile and nonvolatile anesthetics examined thus far augment the activity of GABA_A receptors. This study demonstrates that four nonvolatile anesthetics, α-chloralose, choral hydrate, trichloroethanol and urethane, enhance the GABA-induced chloride conductance of GABA_A receptors expressed in X. laevis oocytes.

Kumamoto and Murata (1996), using a single concentration, demonstrated that α-chloralose (50 μM) augmented the GABA-induced (10 μM) response in dissociated septal neurons. We have extended these observations using the X. laevis oocyte expression system and found that the potentiation by α-chloralose on GABA-induced currents is concentration dependent and the EC_{50} of α-chloralose is within the range observed in the plasma during anesthesia (Nattel et al., 1990). Moody et al. (1988) showed that α-chloralose inhibited[^25]S]-butylbicyclophosphorothionate binding to GABA_A receptors in cortical membranes; however, the IC_{50} was almost 10-fold greater than the EC_{50} value for enhancement of the GABA response observed in the present study. We observed no effect on currents in the absence of GABA at concentrations of α-chloralose up to 10 mM. The effects of α-chloralose reported in the present study are similar to those of inhalation anesthetics, which only enhance the GABA-induced currents and have no direct effect on GABA_A receptors expressed in X. laevis oocytes (Lin et al., 1992).

Several studies using neuronal preparations reported direct effects of α-chloralose. High concentrations of α-chloralose caused a direct increase in current in cultured septal neurons (0.5 mM; Kumamoto and Murata, 1996), frog motoneurons (2 mM; Nicoll and Wojtowicz, 1980) and frog dorsal root ganglia (0.1–20 mM; Ishizuka et al., 1989). One explanation for the difference between our study and those in neuronal cells could be due to differences in the effects of α-chloralose on different subtypes of GABA_A receptors. The receptor subunits present in the neuronal preparations may not be the same as those expressed in our study; however, Harris et al. (1995) have shown that subunit composition has very little effect on the modulation of GABA_A receptor activity by volatile anesthetics. Further studies are needed to determine whether the effects of α-chloralose are dependent on subunits present in the complex. Another possible reason for the incongruity of the α-chloralose effects is that differences between the membrane composition of the X. laevis oocyte and neuronal membranes may affect the function of GABA_A receptors. The direct effects of α-chloralose observed in neuronal preparations could also be due to potentiation of low concentrations of GABA present in the neuronal preparations. GABA release has been reported in cultured hippocampal and thalamic cells (Valeyev et al., 1993; Liu et al., 1995). Blockade of the direct effect of α-chloralose by the competitive GABA antagonist bicuculline in frog motoneurons (Nicoll and Wojtowicz, 1980) and cultured septal neurons (Kumamoto and Murata, 1996) lends support to this hypothesis.

The mechanism of the α-chloralose response is similar to that of other allosteric modulators. α-Chloralose potentiates the GABA-induced current by increasing the affinity for GABA, an attribute common with enflurane, benzodiazepines, neurosteroids and barbiturates (Mihic et al., 1994; Nicoll and Wojtowicz, 1980; Turner and Simmonds, 1989; Sigel and Baur, 1988; Parker et al.). The reversal potential for the α-chloralose response is similar to the chloride equilibrium potential in X. laevis oocytes (−14 mV; Kusano et al., 1982) and shows a chloride dependence that follows the Nernst relationship. This strongly suggests that the enhancement of the GABA-induced inward current is mediated by increased chloride conductance.

Although the pharmacological properties of α-chloralose are similar to those of other allosteric modulators of GABA_A receptors, results from this study indicate that this compound binds to a site distinct from other well-characterized allosteric sites on the GABA_A receptor complex. This observation is based on three findings. First, α-chloralose does not act through the benzodiazepine site on the complex because flumazenil does not block the effects of α-chloralose. Second,
α-chloralose does not bind to the neurosteroid site because 3β-OH-DHP, a neurosteroid partial agonist, inhibits the effects of the neurosteroid agonist 3α-OH-DHP but does not alter the α-chloralose response.

Third, α-chloralose does not bind to the barbiturate site because α-chloralose did not affect the pentobarbital potentiation of GABA-induced currents. Under the conditions of our experiment, it would be expected that α-chloralose would antagonize the effects of pentobarbital if α-chloralose were a partial agonist at the barbiturate binding site. Therefore, the lack of inhibition argues against a common binding site. Although α-chloralose did not inhibit the pentobarbital response, the combination of the two compounds did not produce additive effects as would be expected by ligands binding to separate allosteric sites. Pentobarbital increases the duration of the open channel time produced by GABA (Study and Barker, 1981; Macdonald et al., 1989). At the EC_{100} of pentobarbital, the open channel time may be maximal and further enhancement by other ligands may not be possible. We have also observed the lack of additive effects with EC_{100} concentrations of pentobarbital and neurosteroids. Enhancement of GABA-induced currents with a combination of 300 μM pentobarbital and 1 μM 3α-OH-DHP was equal to the effects of 300 μM pentobarbital alone (data not shown). Overall, these data suggest that α-chloralose and pentobarbital bind to separate sites on the complex; however, complex interactions between these two classes of compounds may occur.

Mihic et al. (1997) recently identified two residues in the second and third transmembrane regions of GABA_3 receptors and glycine receptors that are critical for enhancement of activity by enflurane, isoflurane and ethanol but not propofol. This suggests that not all general anesthetics bind to the same site on the GABA_A receptor complex. It would be of interest to determine whether α-chloralose binds to the same residues as enflurane and isoflurane.

Three other anesthetics also were shown to enhance GABA-induced current. Chloral hydrate is frequently used as a sedative in pediatric patients and as anesthetic in laboratory animals, but little is known about its mechanism of action. We found that chloral hydrate also potentiates GABA-induced chloride conductance at 1 and 10 mM. Chloral hydrate is rapidly metabolized to trichloroethanol and trichloroacetic acid (Butler, 1948). Plasma levels of chloral hydrate are undetectable at doses used for sedation (Breimer, 1977); however, plasma concentrations of 1 to 2 mM have been measured during anesthesia in dogs (Butler, 1948). Thus, it is possible that potentiation of GABA-induced Cl^- currents may be involved, in part, in the initial anesthetic effects of chloral hydrate. Because chloral hydrate is rapidly metabolized, it is thought that trichloroethanol plays a major role in the pharmacological actions of chloral hydrate (Hobbs et al., 1995).

Trichloroethanol significantly augments GABA-evoked responses at 1 mM and plateaus at 10 mM. Trichloroethanol has also been shown to potentiate GABA-induced currents in hippocampal neurons at these concentrations (Loving et al., 1993; Peoples and Weight, 1994). The EC_{50} value of trichloroethanol found to modulate GABA_A receptor activity in vitro is within the range observed during anesthesia in dogs (Butler, 1948).

Urethane, an anesthetic commonly used in the laboratory, alone or in combination with α-chloralose, potentiated GABA-induced Cl^- conductance at 30 and 100 mM. These concentrations are similar to those reported by Moody et al. (1988) to increase Cl^- flux in cortical synaptoneuroses and inhibit [35S]butylbicyclophosphorothionate binding in cortical membranes. However, the minimal effective concentration of urethane that modulates GABA_A activity in vitro is well above the plasma levels found during anesthesia (10 mM; Maggi and Meli, 1986). Therefore, the effects on GABA_A receptors do not appear to be relevant to the anesthetic action (Franks and Lieb, 1994).

Ethanol, butanol, halothane and isoflurane modulate 5-HT_3 receptors in addition to enhancing GABA_A receptor activity (Machu and Harris, 1994). α-Chloralose, at concentrations up to 1 mM, had no effect on the 5-HT_3 receptor. These data suggest that different classes of ligand-gated channels may have different structural requirements for the anesthetics. Volatile anesthetics also potentiate glycine receptors (Harrison et al., 1993), weakly inhibit the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid subtype of glutamate receptors (Lin et al., 1993b; Harris, et al., 1995) and potentiate the kainate subtype of glutamate receptors (Harris et al., 1995). These data suggest that the array of activity of a given anesthetic may depend on the ensemble of channels that the anesthetic affects. Further studies are needed to determine whether the effects of α-chloralose are specific to GABA_A receptors or may be generalized to other members of the family of ligand-gated channels. This study shows that several nonvolatile anesthetics with structures distinct from the volatile anesthetics enhance GABA_A receptor activity, suggesting that allosteric modulation of GABA_A receptors may be a common mechanism of action across broad classes of general anesthetics.

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References


1998 α-Chloralose and GABA_A Receptors


Prince RJ and Simmonds MA (1993) Differential antagonism by epipreganolone of alphaxalone an pregnanolone potentiation of [3H]Dunitezepam binding suggests more than one class of binding site for steroids at GABA<sub>A</sub> receptor. Neuropharmacology 32:59–63.


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