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# Differential Subunit Dependence of the Actions of the General Anesthetics Alphaxalone and Etomidate at γ-Aminobutyric Acid Type A Receptors Expressed in *Xenopus laevis* Oocytes

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## SUMMARY

The effects of subunit composition of the γ-aminobutyric acid (GABA) type A receptor on the multiple actions of the general anesthetics alphaxalone and etomidate were investigated. The abilities of the two drugs to activate directly $Cl^-$ currents and to modulate GABA-evoked $Cl^-$ currents mediated by human recombinant $GABA_A$ receptors composed of α1, γ2S, and either β1, β2, or β3 subunit expressed in *Xenopus laevis* oocytes were compared. Both alphaxalone and etomidate evoked $Cl^-$ currents in α1β1γ2S, α1β2γ2S, and α1β3γ2S receptors, an action that was blocked by both SR 95531 and picrotoxin. However, although maximal current activation by alphaxalone varied only slightly with the specific β subunit isoform present, the efficacy of etomidate showed a rank order of β3 > β2 ⋙ β1. In addition, β1 homomeric receptors were markedly activated by etomidate but not by alphaxalone. Conversely, receptors consisting of α1 and γ2S subunits were markedly activated by alphaxalone but not by etomidate. The modulatory effect of alphaxalone was also not markedly influenced by the β-specific subunit isoform, whereas the modulatory efficacy of etomidate showed a rank order of β3 > β2 ≫ β1. These results further demonstrate that the actions of general anesthetics at $GABA_A$ receptors are influenced by receptor subunit composition, and they suggest that the effects of alphaxalone and etomidate are mediated by different binding sites on the receptor complex.

A wide variety of chemically diverse compounds, including barbiturates, steroids, propofol, alcohols, and inhalation agents such as halothane, isoflurane, and enflurane, induce general anesthesia in animals and humans (1). Biochemical and electrophysiological studies have shown that all of these drugs potentiate the inhibitory signals mediated by $GABA_A$ receptors in the brain and that their potencies and efficacies in exerting this action correlate with their abilities to induce anesthesia (2–7). These observations support the notion that $GABA_A$ receptors play an important role in anesthesia, although the precise molecular mechanism of action of general anesthetics at $GABA_A$ receptors remains to be fully elucidated.

In addition to facilitating the action of GABA at $GABA_A$ receptors, general anesthetics elicit a GABA-like direct effect that can be detected by an increase in $Cl^-$ channel permeability or $^{36}Cl^-$ uptake into brain membrane vesicles in the absence of GABA (8–13). Although this effect occurs at pharmacologically relevant concentrations, they are generally higher than those required to potentiate GABA responses.

Molecular cloning studies have identified a variety of $GABA_A$ receptor subunits and demonstrated receptor population heterogeneity (14), suggesting that the actions of anesthetics, as well as those of other GABAergic modulators, may be influenced by receptor subunit composition. Indeed, Lin *et al*. (15) showed that enflurane potentiated GABA-induced currents to a greater extent at α1β1 than at α1β1γ2 receptors, indicating that the degree of potentiation produced by enflurane can be altered by the γ2 subunit and that at variance with benzodiazepines (16, 17), the modulatory effect does not require this subunit. Similar results have been obtained with other anesthetics, such as isoflurane (18), pentobarbital (19), and propofol (20). We have previously shown that both propofol and pentobarbital activate currents directly and potentiate GABA-induced currents in *Xenopus laevis* oocytes expressing β1 homomeric receptors (13). In addition, Cestari *et al*. (21) reported that pentobarbital directly activates $Cl^-$ currents at homomeric receptors composed of murine β3, but not of β2, subunits expressed in oocytes; chimeras of the two β isoforms revealed that the difference in responsiveness is attributable to a three-amino acid difference in the amino-terminal domains of the two subunits. The influence of β subunits was also demonstrated by Harris *et al*. (22), who showed that positive modulation of $GABA_A$ receptors by the injectable anesthetics alphaxalone and pentobarbital, but not by the volatile anesthetics isoflurane and enflurane, strictly depended on the coexpression of β2 or β3 subunits in transfected cell lines.

**ABBREVIATIONS:** GABA, γ-aminobutyric acid; $GABA_A$, γ-aminobutyric acid type A; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

We have now investigated further the influence of $\beta$ subunit isoforms ($\beta$1, $\beta$2, or $\beta$3), coexpressed with human $\alpha$1 and $\gamma$2S subunits in *X. laevis* oocytes, on the actions of the anesthetics alphaxalone and etomidate. Because we previously showed that alphaxalone does not activate $\beta$1 homomeric receptors directly (13), we predicted that this steroid derivative might be representative of a class of anesthetics that act at sites localized on subunits other than the $\beta$ subunit. On the other hand, preliminary studies in our and other laboratories (23, 24) have shown that both direct and modulatory effects of etomidate are dependent on the type of $\beta$ subunit isoform expressed. In addition, by expressing an array of homomeric and dimeric receptor combinations, we attempted to determine whether specific subunits are required for the actions of these anesthetics.

## Experimental Procedures

**Materials.** Adult *X. laevis* females were obtained from Dipl.Biol.-Dipl.Ing. Horst Kähler (Hamburg, Germany). Alphaxalone and etomidate were kindly provided by Glaxo Group Research (Greenford, UK) and Janssen Pharmaceutica (Beerse, Belgium), respectively; stock solutions (10 mM) were prepared in dimethylsulfoxide and stored at −20° until use. SR 95531 was obtained from Research Biochemicals International (Natick, MA), and GABA, picrotoxin, and other reagents of analytical grade were from Sigma Chemical (St. Louis, MO).

**Preparation of cDNAs.** The cDNAs encoding the human $\alpha$1, $\beta$1, $\beta$2, $\beta$3, and $\gamma$2S $GABA_A$ receptor subunits were subcloned into the pCDM8 expression vector (InVitrogen, San Diego, CA) (25). Plasmids were purified with the Promega Wizard Plus Miniprep DNA Purification System (Madison, WI) and then resuspended in sterile distilled water, divided into portions, and stored at −20° until used for injection.

**Microinjection of and electrophysiological recording from *X. laevis* oocytes.** Oocyte isolation and cDNA microinjection were performed essentially as previously described (20). Isolated oocytes were placed in modified Barth's saline [containing 88 mM NaCl, 1 mM KCl, 10 mM HEPES-NaOH, pH 7.5, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 0.91 mM $CaCl_2$, and 0.33 mM $Ca(NO_3)_2$]. Various mixtures of $GABA_A$ receptor subunit cDNAs (1.5 ng of each in a total volume of 30 nl) were injected into the nucleus of oocytes according to the "blind" method. The injected oocytes were cultured at 19° in sterile modified Barth's saline supplemented with 10 $\mu$g/ml streptomycin, 10 units/ml penicillin, 50 $\mu$g/ml gentamicin, 0.5 mM theophylline, and 2 mM sodium pyruvate. Recordings were obtained 1–4 days after injection from oocytes placed in a 100-$\mu$l rectangular chamber. The animal pole of oocytes was impaled with two glass electrodes (0.5–3 MΩ) filled with 3 M filtered KCl, and the voltage was clamped at −70 mV with an Axoclamp 2-B amplifier (Axon Instruments, Burlingame, CA). Currents were continuously recorded on a strip-chart recorder. Resting membrane potential usually varied between −30 and −50 mV. Drugs were perfused for 20 sec unless otherwise noted. Intervals of 5 min were allowed between applications of low concentrations of GABA alone and of ≥10 min when high concentrations of GABA or anesthetics were applied.

**Statistical analysis.** Currents were expressed as a percentage of the control response (in nA) obtained with GABA alone. A GABA control response was obtained before and after each drug application to take into account possible shifts in the control currents. Oocytes from at least two frogs were used for each experiment, and the total number of oocytes is given. Data are presented as mean ± standard error and were analyzed by Student's $t$ test or by one- or two-way analysis of variance followed by Scheffé's *post hoc* test. $p < 0.05$ was considered statistically significant.

## Results

**Direct effects of alphaxalone and etomidate at $GABA_A$ receptors.** As expected, alphaxalone and etomidate induced inward $Cl^-$ currents in the absence of GABA in oocytes expressing human $GABA_A$ receptors composed of $\alpha1\beta2\gamma2S$ subunits (Fig. 1). Responses to both anesthetics were concentration dependent and reversible; a typical slow current decay was observed, which was most marked at the highest concentrations of these drugs. $Cl^-$ currents induced by either anesthetic were inhibited by the coapplication of either the GABA competitive antagonist SR 95531 or the $Cl^-$ channel blocker picrotoxin (Fig. 2); total inhibition was apparent at a 25 $\mu$M concentration of either SR 95531 or picrotoxin (data not shown).

**Role of $\beta$ subunits in the direct actions of alphaxalone and etomidate.** To evaluate the influence of $\beta$ subunits on the direct activation of the receptor-associated $Cl^-$ conductance by alphaxalone and etomidate, we injected oocytes with cDNAs encoding $\alpha$1 and $\gamma$2S subunits together with those encoding $\beta$1, $\beta$2, or $\beta$3 subunits. Alphaxalone activated $Cl^-$ currents at all receptors with similar efficacies (Fig. 3A), although the maximal effect tended to be greatest at receptors containing the $\beta$1 subunit and smallest at those containing the $\beta$3 subunit (Table 1). In addition, the potency of alphaxalone at $\beta$1-containing $GABA_A$ receptors was approximately twice that at the other two receptor subtypes (Table 1).

Etomidate also activated all receptor subtypes tested (Fig. 3B). However, etomidate showed a markedly lower efficacy and potency at receptors containing the $\beta$1 subunit than at those containing the $\beta$2 or $\beta$3 subunits (Table 1).

Comparison of the direct actions of alphaxalone and etomidate at the various receptor subunit assemblies reveals that the efficacy of etomidate at $\alpha1\beta2\gamma2S$ and $\alpha1\beta3\gamma2S$ receptors is approximately twice that of alphaxalone, although its potency is only one half that of the steroid anesthetic. In contrast, at receptors containing the $\beta$1 subunit, etomidate was less effective than alphaxalone and showed one twentieth of the potency of alphaxalone (Table 1).

**Direct actions of alphaxalone and etomidate at homomeric and dimeric $GABA_A$ receptors.** To investigate whether specific subunits of the $GABA_A$ receptor are required for the direct activation of $Cl^-$ currents by the two anesthetics, we compared the actions of these compounds at $\beta$1 homomeric receptors and at receptors formed from two or three different subunits.

As shown previously (13), alphaxalone failed to induce substantial $Cl^-$ currents at $\beta$1 homomeric receptors. However, coexpression of this subunit with $\alpha$1 or $\gamma$2S subunits restored sensitivity of the receptor to alphaxalone (Fig. 4A); maximal activation at $\alpha1\beta1$ receptors was twice that at $\beta1\gamma2S$ receptors. Of all subunit assemblies tested, including $\alpha1\beta1\gamma2S$, the effect of alphaxalone was greatest at receptors composed of $\alpha$1 and $\gamma$2S subunits.

Unlike alphaxalone, etomidate markedly activated $Cl^-$ currents at $\beta$1 homomeric receptors (Fig. 4B), resembling in this respect the general anesthetics pentobarbital and propofol (13). Coexpression of $\alpha$1 with the $\beta$1 subunit resulted in a marked reduction in the efficacy of etomidate, whereas coexpression of $\gamma$2S with $\beta$1 had no effect on the efficacy of this drug (Fig. 4B). Receptors composed of $\alpha$1 and $\gamma$2S subunits

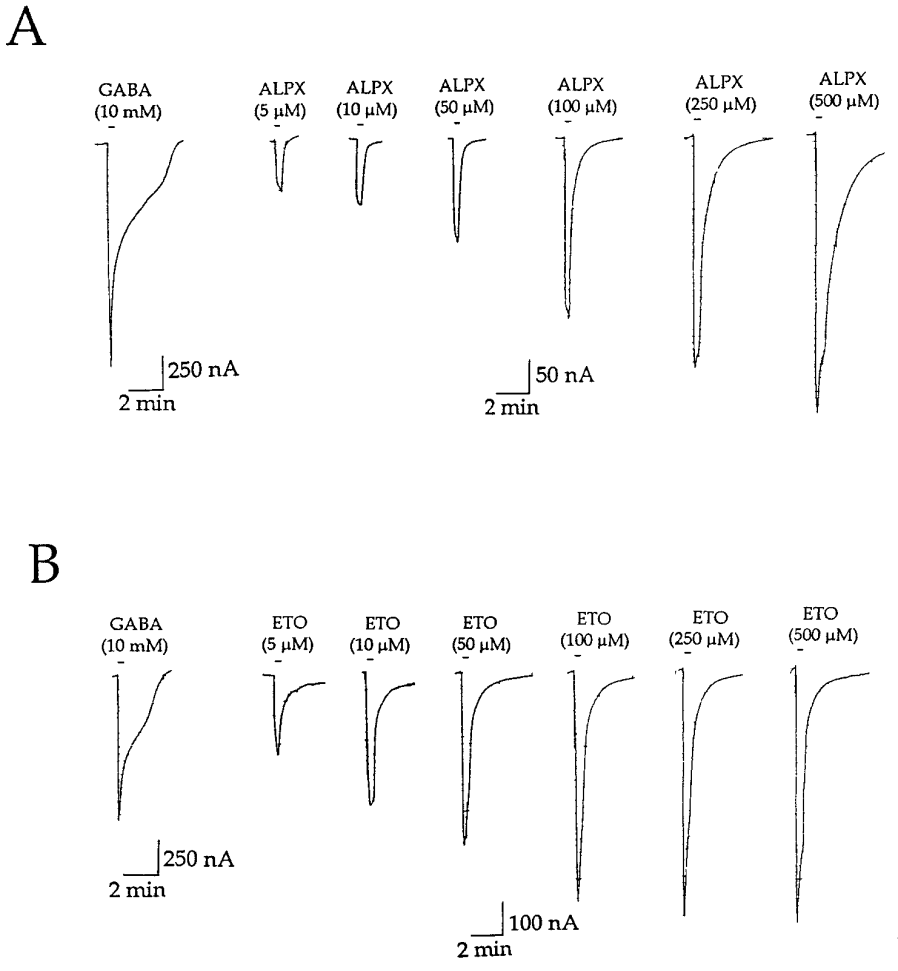


**Fig. 1.** Direct activation of human recombinant $GABA_A$ receptors by alphaxalone (*ALPX*) (A) and etomidate (*ETO*) (B). *Tracings* were obtained from single oocytes expressing $\alpha1\beta2\gamma2S$ receptors and represent $Cl^-$ currents induced by the two anesthetics in the absence of GABA compared with the response to 10 mM GABA. *Horizontal bar above each response*, drug application (20 sec).

were not substantially activated by etomidate, suggesting that in contrast to alphaxalone, the $\beta1$ subunit is required for the GABA-mimetic action of this anesthetic. Coexpression of $\alpha1$ and $\gamma2S$ subunits with the $\beta1$ subunit also markedly reduced the sensitivity of the latter subunit to etomidate.

**Role of $\beta$ subunits in the modulation of GABA-induced currents by alphaxalone and etomidate.** Both anesthetics have previously been shown to enhance markedly the action of GABA at $GABA_A$ receptors (9, 10, 26–28). Concentration-response curves for the modulatory effect of alphaxalone (0.1–100 $\mu$M) on $Cl^-$ currents induced by GABA (20% of maximally effective concentration) revealed similar efficacies at $\alpha1\beta1\gamma2S$, $\alpha1\beta2\gamma2S$, and $\alpha1\beta3\gamma2S$ receptor subtypes (Fig. 5A, Table 2). However, the potency of alphaxalone at receptors containing the $\beta2$ subunit was approximately two to three times that at the other two subunit assemblies.

Maximal enhancement of GABA-induced currents by etomidate at $\beta3$-containing receptors was slightly (not statistically different) and markedly greater than that at receptors containing $\beta2$ and $\beta1$, respectively (Fig. 5B, Table 2). The potency of etomidate in modulating GABA-induced currents at $\alpha1\beta1\gamma2S$ receptors was one seventh to one third that at the other two receptor subtypes.

Although the maximal enhancement of GABA-induced currents by alphaxalone and etomidate was similar at $\beta2$- and $\beta3$-containing receptors, etomidate was less effective than alphaxalone at receptors containing the $\beta1$ subunit. In addi-

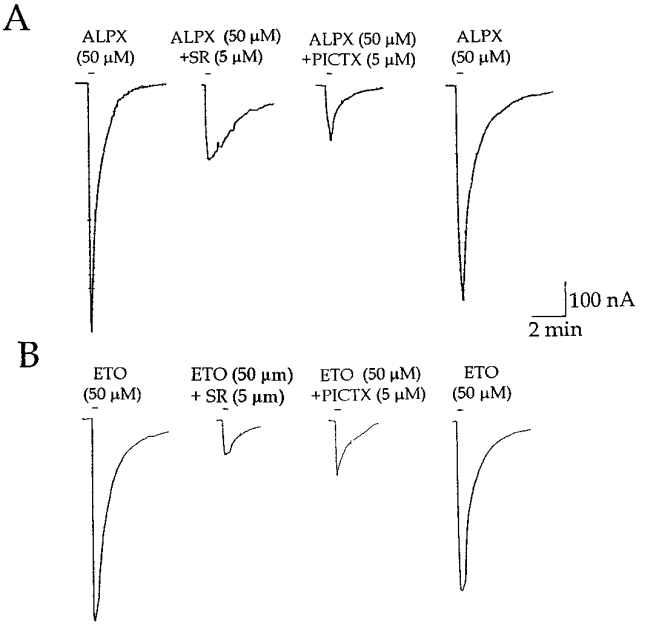


**Fig. 2.** Effects of SR 95531 and picrotoxin on $GABA_A$ receptor-mediated $Cl^-$ currents induced by alphaxalone (*ALPX*) (A) and etomidate (*ETO*) (B). *Tracings* were obtained from single oocytes expressing $\alpha1\beta2\gamma2S$ receptors and represent the inhibition of the responses to the two anesthetics by 5 $\mu$M SR 95531 (*SR*) and 5 $\mu$M picrotoxin (*PICTX*). Drugs were perfused for 20 sec.

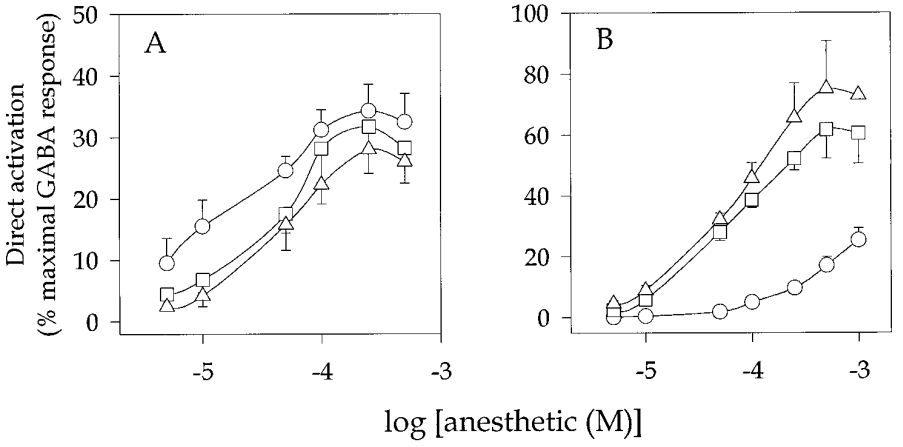


**Fig. 3.** Direct effects of alphaxalone (A) and etomidate (B) at $GABA_A$ receptor assemblies containing different $\beta$ subunit isoforms. Evoked $Cl^-$ currents were in response to various concentrations of the two anesthetics measured from oocytes expressing $\alpha1\beta1\gamma2S$ (○), $\alpha1\beta2\gamma2S$ (□), or $\alpha1\beta3\gamma2S$ (△) receptors. Values are expressed as mean ± standard error percentage of the control response obtained with 10 mM GABA (from five to seven oocytes).

TABLE 1

**Direct activation of $Cl^-$ currents by alphaxalone and etomidate in *X. laevis* oocytes expressing recombinant $GABA_A$ receptors**

Maximal direct activation of $Cl^-$ current is expressed as a percentage of the control response obtained with 10 mM GABA; values are mean ± standard error for the indicated number (*n*) of oocytes.

| Receptor construct | *n* | Maximal activation | $EC_{50}$ |
|---|---|---|---|
| | | % | μM |
| Alphaxalone | | | |
| $\alpha1\beta1\gamma2S$ | 7 | 34 ± 2 | 15[a] |
| $\alpha1\beta2\gamma2S$ | 5 | 32 ± 4 | 31 |
| $\alpha1\beta3\gamma2S$ | 5 | 28 ± 4 | 37 |
| Etomidate | | | |
| $\alpha1\beta1\gamma2S$ | 5 | 25 ± 4[a] | 319[a] |
| $\alpha1\beta2\gamma2S$ | 5 | 62 ± 10 | 67 |
| $\alpha1\beta3\gamma2S$ | 5 | 75 ± 16 | 70 |

[a] $p < 0.01$ ($\alpha1\beta1\gamma2S$ versus $\alpha1\beta2\gamma2S$ or $\alpha1\beta3\gamma2S$ receptor construct; Student's *t* test).

tion, both anesthetics showed higher potency at $\alpha1\beta2\gamma2S$ receptors than at the other two receptor subtypes.

**Modulation of homomeric and dimeric $GABA_A$ receptors by alphaxalone and etomidate.** Alphaxalone and etomidate each potentiated $Cl^-$ currents evoked by GABA (20% of maximally effective concentration) to a similar extent in oocytes expressing $\beta1$, $\alpha1\beta1$, $\beta1\gamma2S$, $\alpha1\gamma2S$, or $\alpha1\beta1\gamma2S$ receptors (Fig. 6). Thus, no single subunit or subunit combination was required for the modulatory action of these anesthetics.

## Discussion

Recent studies have suggested that the $\beta$ subunit of the $GABA_A$ receptor may be required for the action of certain anesthetics (13, 20–22). Thus, we focused our attention on the role of this subunit in both the GABA-mimetic and modulatory actions of alphaxalone and etomidate at recombinant human $GABA_A$ receptors expressed in *X. laevis* oocytes. Our results show that the two anesthetics differ markedly in the subunit dependence, especially the $\beta$ subunit sensitivity, of their multiple actions.

Alphaxalone and etomidate have previously been shown to exert a GABA-mimetic action in the absence of GABA (9, 10, 13, 29). Here, we demonstrate that in addition to $\alpha1\beta x\gamma2S$ receptors, alphaxalone activates receptors formed by $\alpha1$ and $\gamma2S$ subunits. In contrast, even high concentrations of this anesthetic exert only a weak activating effect at $\beta1$ homomeric receptors, as previously shown (13). These results suggest that this steroid anesthetic interacts preferentially with $\alpha1$ and $\gamma2S$ subunits but not with the $\beta1$ subunit. In contrast, Puia *et al.* (27) showed that human $\beta1$ homomeric receptors expressed in human embryonic kidney 293 cells were directly activated by the steroid derivatives 3α,21-dihydroxy-5α-pregnan-20-one and 3α-hydroxy-5α-pregnan-20-one. Differences in potency or efficacy between these endogenous steroids and alphaxalone may account for this apparent discrepancy. In addition, because absolute values for steroid-induced currents were provided in the previous study, whereas we expressed alphaxalone-induced currents as a percentage of the maximal GABA response in the current study, the results of the two studies are not readily compared.

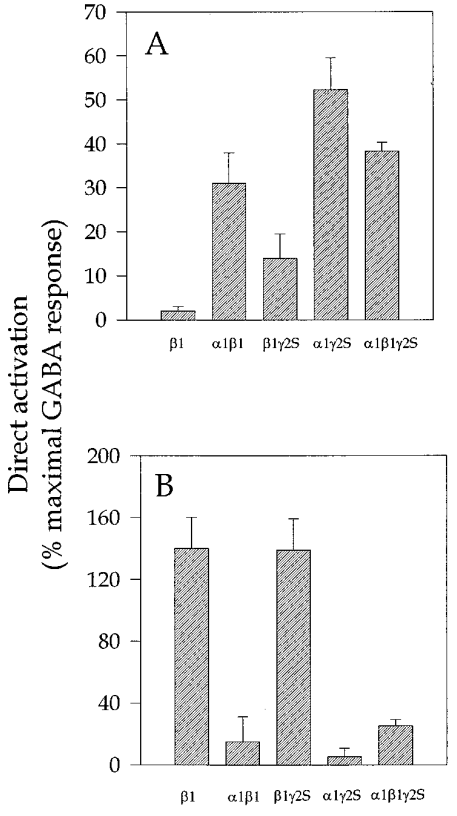


**Fig. 4.** Direct actions of alphaxalone (A) and etomidate (B) at homomeric, dimeric, and trimeric $GABA_A$ receptor assemblies. Values represent the $Cl^-$ currents induced by the two anesthetics in the absence of GABA and are expressed as mean ± standard error percentage of the control response obtained with 10 mM GABA (from 5–10 oocytes).

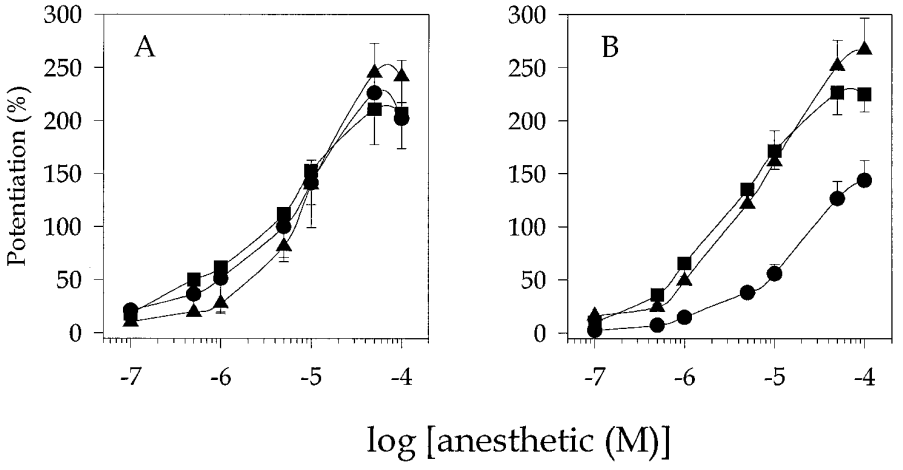


**Fig. 5.** Modulatory actions of alphaxalone (A) and etomidate (B) at $GABA_A$ receptor assemblies containing different $\beta$ subunit isoforms. Values are expressed as mean ± standard error percentage of the potentiation of the control response to GABA ($EC_{20}$) by various concentrations of the two anesthetics measured in oocytes expressing $\alpha1\beta1\gamma2S$ (●), $\alpha1\beta2\gamma2S$ (■), or $\alpha1\beta3\gamma2S$ (▲) receptors (from six to nine oocytes). Actual $EC_{20}$ concentrations of GABA for the different receptor combinations were determined experimentally for each oocyte: $\alpha1\beta1\gamma2S$, 5–15 $\mu$M (mean, 9.3 ± 0.9 $\mu$M); $\alpha1\beta2\gamma2S$, 2–10 $\mu$M (mean, 5.4 ± 0.9 $\mu$M); and $\alpha1\beta3\gamma2S$, 1–10 $\mu$M (mean, 3.5 ± 0.6 $\mu$M).

TABLE 2

**Modulation by alphaxalone and etomidate of GABA-induced $Cl^-$ currents in *X. laevis* oocytes expressing recombinant human $GABA_A$ receptors**

Maximal potentiation is expressed as the percentage increase in the current induced by GABA at $EC_{20}$. Data are mean ± standard error for the indicated number (*n*) of oocytes.

| Receptor construct | *n* | Maximal potentiation | $EC_{50}$ |
|---|---|---|---|
| | | % | $\mu$M |
| Alphaxalone | | | |
| $\alpha1\beta1\gamma2S$ | 7 | 226 ± 17 | 7.1 |
| $\alpha1\beta2\gamma2S$ | 6 | 211 ± 33 | 2.8 |
| $\alpha1\beta3\gamma2S$ | 6 | 245 ± 25 | 9.7 |
| Etomidate | | | |
| $\alpha1\beta1\gamma2S$ | 9 | 144 ± 19[a] | 26[a] |
| $\alpha1\beta2\gamma2S$ | 9 | 227 ± 20 | 3.6 |
| $\alpha1\beta3\gamma2S$ | 9 | 267 ± 29 | 8.6 |

[a] $p < 0.01$ ($\alpha1\beta1\gamma2S$ versus $\alpha1\beta2\gamma2S$ or $\alpha1\beta3\gamma2S$ receptor construct; Student's *t* test).

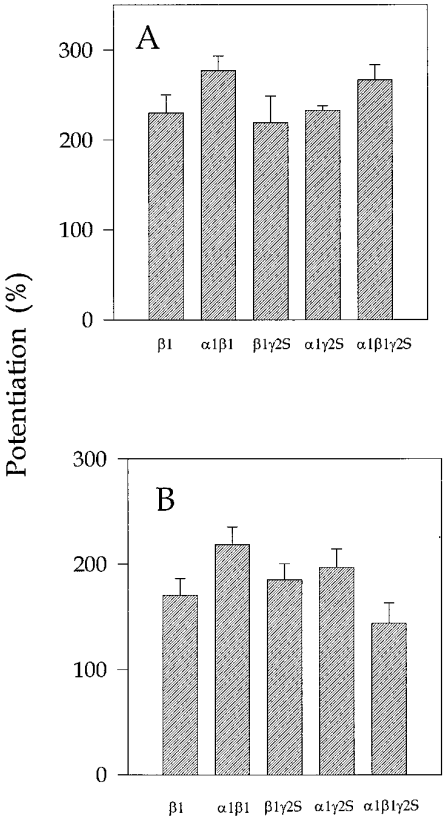


**Fig. 6.** Modulatory actions of alphaxalone (A) and etomidate (B) at homomeric, dimeric, and trimeric $GABA_A$ receptor assemblies. Values represent the potentiation of $Cl^-$ currents induced by GABA by the two anesthetics and are expressed as mean ± standard error percentage increase in the control response obtained with GABA ($EC_{20}$) (from 5–10 oocytes). $EC_{20}$ concentrations of GABA for the different receptor combinations were determined experimentally for each oocyte: $\beta1$, 15–32 $\mu$M (mean, 22.3 ± 2 $\mu$M); $\alpha1\beta1$, 0.5–6 $\mu$M (mean, 2.1 ± 0.4 $\mu$M); $\beta1\gamma2S$, 10–30 $\mu$M (mean, 16.8 ± 2 $\mu$M); $\alpha1\gamma2S$, 5–20 $\mu$M (mean, 8.6 ± 2 $\mu$M); and $\alpha1\beta1\gamma2S$, 5–15 $\mu$M (mean, 9.3 ± 0.9 $\mu$M).

Coexpression of either $\alpha1$ or $\gamma2S$ subunits with the $\beta1$ subunit restored sensitivity of the resulting receptors to alphaxalone. However, currents elicited by this anesthetic were lower at $\alpha1\beta1$, $\beta1\gamma2S$, and $\alpha1\beta1\gamma2S$ receptor constructs than at those composed of only $\alpha1$ and $\gamma2S$ subunits. These observations suggest that in addition to generating functional GABA-sensitive $Cl^-$ channels (30), both $\alpha1$ and $\gamma2S$ subunits contribute to the formation of a sensitive site for the interaction of alphaxalone and that the presence of the $\beta1$ subunit reduces the sensitivity of the receptor to this anesthetic. Furthermore, alphaxalone sensitivity seems to require the presence of at least two different types of subunits, one of which must be either $\alpha1$ or $\gamma2S$. The notion that the $\beta$ subunit may not be important in the direct action of alphaxalone is also supported by the observation that the anesthetic showed similar efficacies at trimeric receptors containing different $\beta$ subunit isoforms, although its potency at $\alpha1\beta1\gamma2S$ receptors was twice that at receptors containing the $\beta2$ or $\beta3$ subunit.

Etomidate differed from alphaxalone in that its direct action at $GABA_A$ receptors required the $\beta$ subunit. Thus, etomidate activated $\beta1$ homomeric receptors with an efficacy higher than that of GABA itself, but it had no effect at $\alpha1\gamma2S$ assemblies. In addition, coexpression of $\alpha1$, but not of $\gamma2S$, markedly reduced the sensitivity of the receptor to etomidate. These observations suggest that unlike alphaxalone, etomidate may interact directly with the $\beta1$ subunit and that the site of action on this subunit is affected in a negative manner by the presence of the $\alpha1$ subunit.

In contrast to alphaxalone, the importance of the $\beta$ subunit in the GABA-mimetic action of etomidate is further supported by the observation that within the range of concentrations tested, the efficacy of this anesthetic depended on

which $\beta$ subunit isoform was expressed together with the $\alpha 1$ and $\gamma 2S$ subunits, with the rank order $\beta 3 > \beta 2 \ggg \beta 1$. It should be noted, however, that the concentration-response curve for etomidate at $\alpha 1\beta 1\gamma S$ receptors does not reach clear saturation, and therefore the actual efficacy of this compound may not be accurately determined. A similar influence of the $\beta$ subunit has been demonstrated for the direct action of pentobarbital (31). Together, these observations indicate that alphaxalone and etomidate directly activate $GABA_A$ receptors by interacting at sites localized on different subunits: the $\beta$ subunit for etomidate and $\alpha 1$ or $\gamma 2S$ subunits for alphaxalone. Together with the results of previous studies (13, 20), the current data indicate that with regard to $\beta$ subunit specificity, the action of etomidate, but not that of alphaxalone, is similar to that of the general anesthetics propofol and pentobarbital.

Despite the fact that alphaxalone and etomidate seem to act at different subunits of the $GABA_A$ receptor, the direct effects of both drugs were blocked by the GABA competitive antagonist SR 95531. This observation is consistent with previous studies showing that the direct actions of these as well as other anesthetics are inhibited by the GABA competitive antagonist bicuculline (8, 9, 12, 20, 26). Given that general anesthetics are thought to be allosteric modulators of $GABA_A$ receptors (1, 5), it is possible that they may induce a conformational change in the heteromeric receptor complex that encompasses the GABA binding site, an event that can be allosterically inhibited by a competitive antagonist. In contrast, Thompson *et al*. (31) reported that SR 95531 failed to block $Cl^-$ currents induced by pentobarbital at $GABA_A$ receptors expressed in oocytes. The reason for this apparent discrepancy is not clear.

As shown previously with both native and recombinant $GABA_A$ receptors (1, 5), both alphaxalone and etomidate markedly potentiate the action of GABA. In contrast to the subunit specificity of the direct actions of alphaxalone and etomidate, potentiation of the GABA effect by these anesthetics did not require any specific subunit. Indeed, as shown previously (13, 27), alphaxalone enhanced GABA-evoked $Cl^-$ currents at $\beta 1$ homomeric receptors, at which the same compound failed to exert a direct effect. Similarly, etomidate potentiated the action of GABA at all receptors tested, including $\alpha 1\gamma 2S$ receptors, which were insensitive to direct activation by this drug. Thus, it seems that the multiple actions of these anesthetics are mediated by different binding sites: one on the $\beta$ subunit or on the $\alpha 1\gamma 2S$ subunits for the direct effect of etomidate and alphaxalone, respectively, and a second, which is present in all receptors, for potentiation of GABA action. The fact that alphaxalone potentiates the action of GABA at $\beta 1$ homomeric receptors but fails to activate directly these same ion channels suggests that the interaction of GABA with its recognition site may unmask the allosteric site responsible for the potentiating effect of this anesthetic. A similar scenario may account for the action of etomidate at $\alpha 1\gamma 2S$ receptors. Multiple sites of action on $GABA_A$ receptors have also been suggested for propofol and pentobarbital (20, 31). Such multiple sites of action may differ in affinity, as suggested by the fact that higher concentrations of anesthetics are required for direct activation than for modulatory action. The efficacy of the modulatory action of etomidate, but not that of alphaxalone, at trimeric receptors depended, as determined with the range of anesthetic concentrations tested, on the specific $\beta$ subunit isoforms present, with the rank order $\beta 3 > \beta 2 \gg \beta 1$, which is consistent with the notion that these anesthetics influence $GABA_A$ receptor function by acting at different modulatory sites.

The relatively low efficacies and potencies of etomidate with regard to both direct and modulatory effects at $\beta 1$-containing receptors are consistent with the subunit specificity of its analog loreclezole, an anticonvulsant compound devoid of anesthetic properties (32) that interacts selectively with a site located on the $\beta$ subunit of the $GABA_A$ receptor (33, 34); the affinity of loreclezole for receptors containing $\beta 2$ or $\beta 3$ subunits is ~300-fold that for $\beta 1$-containing receptors. The physiological and pharmacological consequences of the marked difference between alphaxalone and etomidate with regard to subunit specificity for their actions at $GABA_A$ receptors, especially at $\beta 1$ homomeric and dimeric receptors, are questionable in view of the fact that such subunit assemblies are unlikely to be expressed as such in neurons (35). However, the expression of single- or double-subunit combinations in *X. laevis* oocytes represents a useful model for evaluation of the role of subunits in the action of general anesthetics. Because etomidate, propofol, and pentobarbital activate $\beta 1$ homomeric receptors, which are regarded as an ancestral form of GABA receptor (36), sensitivity to these compounds and their physiological counterparts probably evolved early during phylogenesis, whereas sensitivity to steroids may have arisen with the appearance of the $\alpha$ and $\gamma 2$ subunits.

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