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Etomidate Effects on Desensitization and Deactivation of $\alpha4\beta3\delta$ $GABA_A$ Receptors Inducibly Expressed in HEK293 TetR Cells

Yiwei Liao, Xiang Liu, Youssef Jounaidi, Stuart A. Forman, and Hua-Jun Feng

Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts (Y.L., X.L., Y.J., S.A.F., H.-J.F.); Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha, China (Y.L.); and Department of Anesthesia, The Sixth Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China (X.L.)

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ABSTRACT

Central $\alpha4\beta\delta$ receptors are the most abundant isoform of δ subunit–containing extrasynaptic $GABA_A$ receptors that mediate tonic inhibition. Although the amplitude of GABA-activated currents through $\alpha4\beta\delta$ receptors is modulated by multiple general anesthetics, the effects of general anesthetics on desensitization and deactivation of $\alpha4\beta\delta$ receptors remain unknown. In the current study, we investigated the effect of etomidate, a potent general anesthetic, on the kinetics and the pseudo steady-state current amplitude of $\alpha4\beta3\delta$ receptors inducibly expressed in human embryonic kidney 293 TetR cells. Etomidate directly activates $\alpha4\beta3\delta$ receptors in a concentration-dependent manner. Etomidate at a clinically relevant concentration (3.2 μM) enhances maximal response without altering the EC_{50} of GABA concentration response. Etomidate also increases the extent of desensitization and prolongs the deactivation of $\alpha4\beta3\delta$ receptors in the presence of maximally activating concentrations of GABA (1 mM). To mimic the modulatory effect of etomidate on tonic currents, long pulses (30–60 seconds) of a low GABA concentration (1 μM) were applied to activate $\alpha4\beta3\delta$ receptors in the absence and presence of etomidate. Although etomidate increases the desensitization of $\alpha4\beta3\delta$ receptors, the pseudo steady-state current amplitude at 1 μM GABA is augmented by etomidate. Our data demonstrate that etomidate enhances the pseudo steady-state current of $\alpha4\beta3\delta$ receptors evoked by a GABA concentration comparable to an ambient GABA level, suggesting that $\alpha4\beta3\delta$ receptors may mediate etomidate's anesthetic effect in the brain.

Introduction

γ-Aminobutyric acid type A ($GABA_A$) receptors are important inhibitory ion channels in the adult mammalian brain (Chua and Chebib, 2017). $GABA_A$ receptors are pentameric chloride ion channels, which are formed from multiple receptor subunit subtypes: $\alpha1$–$\alpha6$, $\beta1$–$\beta3$, $\gamma1$–$\gamma3$, δ, ε, π, and θ (Olsen and Sieghart, 2008). The $\alpha\beta\delta$ receptors localize extrasynaptically and regulate GABAergic tonic inhibition by responding to low ambient GABA concentrations (Mody and Pearce, 2004; Farrant and Nusser, 2005; Feng and Forman, 2018). The most abundant δ subunit–containing $GABA_A$ receptors in the central nervous system are $\alpha4\beta\delta$ receptors (McKernan and Whiting, 1996), although only 7% of $\alpha4$ subunit–containing receptors contain the δ subunit (Bencsits et al., 1999). Compared with $\alpha\beta\gamma$ receptors that are located in synapses and mediate phasic inhibition, $\alpha\beta\delta$ receptors exhibit very low GABA efficacy and slower desensitization (Feng, 2010; Feng and Forman, 2018). $GABA_A$ receptor kinetic properties, including desensitization and deactivation, contribute significantly to shaping phasic GABAergic responses (Jones and Westbrook, 1995; Bianchi et al., 2001). Similar modulator effects on tonic $\alpha\beta\delta$ receptor currents will primarily reflect the balance of drug effects on activation versus desensitization (Liu et al., 2015).

Etomidate is a potent general anesthetic. At clinically relevant concentrations (3.2 μM, twice the EC_{50} for loss of righting reflexes in tadpoles), etomidate enhances GABA activation of $GABA_A$ receptors, and at higher concentrations, it can directly activate $GABA_A$ receptors (Rüsch et al., 2004). Etomidate evokes apparently divergent effects on GABA concentration responses in $\alpha1\beta3\delta$ and $\alpha1\beta3\gamma2$ receptors; however, quantitative modeling analysis indicates that etomidate exerts similar effects on channel gating in both receptor isoforms (Feng et al., 2014). Because etomidate selectively binds to β^+/α^- transmembrane intersubunit sites (Forman and Miller, 2016), these data support the idea that the stoichiometry and subunit arrangement of $\alpha1\beta\delta$ receptors are similar to those of $\alpha1\beta\gamma2$ receptors (Botzolakis et al., 2016). Etomidate reduces the extent of desensitization of concatenated $\beta3$-$\alpha1$-δ/$\beta3$-$\alpha1$ receptors, which have the same stoichiometry and subunit arrangement as $\alpha1\beta3\gamma2$ receptors (Liu et al., 2015). A recent photolabeling study indicated that the subunit arrangement of $\alpha4\beta3\delta$ receptors that are inducibly expressed in human embryonic kidney 293 (HEK293) TetR

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ABBREVIATIONS: HEK, human embryonic kidney.

cells may include $\beta3^{+}/\beta3^{-}$ interfaces, possibly in the form of $\beta3$-$\beta3$-δ-$\beta3$-$\alpha4$ or $\beta3$-$\beta3$-$\alpha4$-δ-$\alpha4$ (Chiara et al., 2016). These data suggest that the stoichiometry and subunit arrangement of $\alpha4\beta3\delta$ receptors may be different from those of $\alpha1\beta3\delta$ receptors. Thus, we hypothesized that etomidate may modulate kinetic properties of $\alpha4\beta3\delta$ receptors differently than those of $\alpha1\beta3\delta$ receptors.

Materials and Methods

Expression of $\alpha4\beta3\delta$ and $\alpha4\beta3$ Receptors. Creation of the HEK293 TetR cell line that inducibly expresses human $\alpha4\beta3\delta$ receptors was described previously (Chiara et al., 2016; Zhou et al., 2018). The $\alpha4\beta3$ receptors were expressed in HEK293T cells using transient transfection (Liu et al., 2015). The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Grand Island, NY) in an incubator with 5% CO_2 and 95% air at 37°C. For the $\alpha4\beta3\delta$ receptor cell line, the growth medium was also supplemented with 250 μg/ml zeocin (Invitrogen, Carlsbad, CA), 5 μg/ml blasticidin, 50 μg/ml hygromycin B, and 200 μg/ml G418 to maintain the expression of genomically integrated cDNAs for $GABA_A$ receptor subunits. For transient transfection of $\alpha4\beta3$ receptors, 2 μg of human $\alpha4$ and $\beta3$ subunit cDNAs with a 1:1 molar ratio was used. pmaxGFP (Amaxa, Gaithersburg, MD) at 0.25 μg was added to each transfection for identification of transfected cells using fluorescence microscopy. Whole-cell electrophysiological recordings were performed 24–48 hours after induction of subunit expression with tetracycline (1 μg/ml) and 5 mM sodium butyrate for the $\alpha4\beta3\delta$ receptor cell line or after transient transfection for $\alpha4\beta3$ receptors.

Whole-Cell Patch-Clamp Recordings. Whole-cell recordings from lifted HEK293 TetR or HEK293T cells were carried out using a fast solution-exchange device at room temperature (Liu et al., 2015). The external solution was composed of (in mM) 142 NaCl, 1 $CaCl_2$, 6 $MgCl_2$, 8 KCl, 10 glucose, and 10 HEPES (pH 7.4), and the internal solution was composed of 153 KCl, 1 $MgCl_2$, 10 HEPES, 5 EGTA, and 2 MgATP (pH 7.3). Recording electrodes, at 1.0–2.0 MΩ, were pulled from borosilicate glasses (TW150F-4; World Precision Instruments, Sarasota, FL) using a P-87 Flaming Brown micropipette puller (Sutter Instruments, Rafael, CA). Cells were voltage-clamped at −50 mV using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) during recordings. Currents were low-pass filtered at 1 kHz and digitized at 2–10 kHz (Digidata 1322A; Molecular Devices), and stored on a personal computer for offline analysis. Series resistance was not compensated. GABA and/or etomidate (Amidate; Hospira, Lake Forest, IL) was delivered via channels in a 2 × 2 quad micropipette that was translated in two orthogonal directions by piezo-electric elements. This solution-exchange device allows for fast switches among solutions in four barrels, with a solution exchange time <2 ms (Liu et al., 2015). The intervals between consecutive drug applications were at least 60 seconds to avoid accumulation of receptor desensitization. For studies on etomidate direct activation and GABA concentration responses in the absence and presence of etomidate, drugs were applied for 4 seconds without preapplication. For studies on kinetic properties, etomidate was preapplied for 2 seconds prior to coapplication of GABA and etomidate for 4 seconds. For studies to mimic the effect of etomidate on tonic currents, etomidate was preapplied for 2 seconds prior to coapplication of GABA and etomidate for 30–60 seconds. The preapplication protocol was not used for concentration-response studies, as the additional solution switching and time required for washout and recovery with this protocol made it difficult to reliably complete concentration-response studies in single cells.

Data Analysis. Whole-cell currents were analyzed using Clampfit 8.2 (Molecular Devices). Percentage of GABA peak current or pseudo steady-state current enhancement by etomidate was calculated by dividing the peak current or pseudo steady-state current elicited by GABA and etomidate coapplication by the peak current or pseudo steady-state current elicited by GABA application alone in the same cell. In concentration responses, peak currents were normalized to those evoked by 0.3 mM etomidate or by 0.3–1 mM GABA, and normalized data for individual cells were fitted using a logistic equation with variable slope: $I = I_{max}/(1 + 10^{(\text{LogEC50} - \text{Log[GABA]}) \times \text{Hill slope}})$. In this equation, I is the normalized peak current in the absence and presence of etomidate, and I_{max} is the maximal normalized GABA current. For preapplication studies, the extent of current desensitization (desensitization percentage) was calculated as a percentage of current reduction (peak current − current at the end of the drug application/peak current). The deactivation phase of whole-cell currents was fitted with single or double exponential decay functions using the Levenberg-Marquardt nonlinear least-squares method. For deactivation with double exponential functions, a weighted time constant (τ_w) was calculated using the formula $\Sigma(ai \times \tau i)/\Sigma ai$ ($i = 2$), in which ai is the fractional amplitude, and τi is the time constant.

Data are reported as the mean ± S.E.M. Statistical analyses were performed using GraphPad Prism 5.0d (GraphPad Software, La Jolla, CA). Unpaired Student's t test was performed to compare GABA EC_{50} in the absence and presence of etomidate for GABA concentration responses of $\alpha4\beta3\delta$ receptors and to compare the peak current enhancement between $\alpha4\beta3\delta$ and $\alpha4\beta3$ receptors. The peak currents and kinetic properties (desensitization and deactivation) of $\alpha4\beta3\delta$ and $\alpha4\beta3$ receptors as well as the pseudo steady-state current amplitudes of $\alpha4\beta3\delta$ receptors prior to and after etomidate treatment were compared using a one-sample t test or paired Student's t test. Statistical significance was inferred at $P < 0.05$.

Results

Etomidate Directly Activates $\alpha4\beta3\delta$ Receptors in a Concentration-Dependent Manner. Etomidate has been shown to directly activate synaptic $GABA_A$ receptors (Rüsch et al., 2004). We examined the effect of etomidate on the function of $\alpha4\beta3\delta$ receptors inducibly expressed in HEK293 TetR cells. Etomidate alone at varied concentrations evoked whole-cell currents in $\alpha4\beta3\delta$ receptors (Fig. 1A), and the direct activation of $\alpha4\beta3\delta$ receptors by etomidate was concentration-dependent (Fig. 1). The EC_{50} of etomidate concentration response was 25 ± 4.5 μM ($n = 6$) (Fig. 1B).

Etomidate Increases Maximal GABA Responses without Altering GABA EC_{50} in $\alpha4\beta3\delta$ Receptors. GABA concentration responses in the absence and presence of 3.2 μM etomidate, a clinically relevant concentration, were assessed in $\alpha4\beta3\delta$ receptors (Fig. 2A). The $\alpha4\beta3\delta$ receptor GABA EC_{50} in the absence of etomidate was 2.3 ± 0.56 μM ($n = 6$), which was consistent with a previous study (Zhou et al., 2018). In the presence of etomidate, the GABA EC_{50} was 1.6 ± 0.50 μM ($n = 8$). The GABA EC_{50} values in the absence and presence of etomidate were not significantly different ($P = 0.39$) (Fig. 2B). Etomidate increased maximal GABA responses (Fig. 2B). The maximal enhancement of GABA current by etomidate was 370% ± 42%, which was significantly different from GABA control ($P < 0.001$).

Etomidate Alters the Desensitization and Deactivation of $\alpha4\beta3\delta$ and $\alpha4\beta3$ Receptors. The effects of etomidate on desensitization and deactivation of $\alpha4\beta3\delta$ receptors were examined by applying a high concentration (1 mM) of GABA in the absence and presence of etomidate (3.2 μM) with etomidate preapplied (Fig. 3A). In line with concentration-response studies, etomidate enhanced the peak currents evoked by 1 mM GABA by 730% ± 113% ($n = 10$;

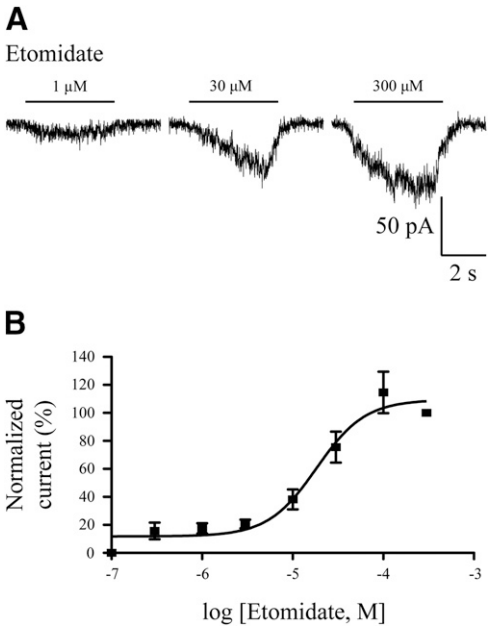

Fig. 1. Etomidate alone concentration-dependently activates $\alpha4\beta3\delta$ receptors. (A) Examples of whole-cell current traces evoked by different concentrations of etomidate from $\alpha4\beta3\delta$ receptors inducibly expressed in HEK293 TetR cells. The solid lines indicate the duration of etomidate application (4 seconds). (B) the concentration-response curve of etomidate alone for $\alpha4\beta3\delta$ receptors. Error bars denote S.E.M.

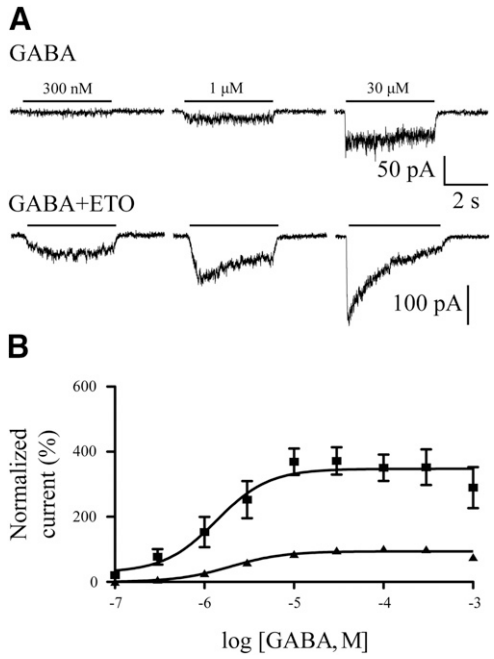

Fig. 2. Etomidate produces an upward shift of GABA concentration responses for $\alpha4\beta3\delta$ receptors. (A) Whole-cell current traces of GABA concentration responses in the absence and presence of etomidate (ETO; 3.2 μM) for $\alpha4\beta3\delta$ receptors inducibly expressed in HEK293 TetR cells. (B) Concentration-response curves of GABA alone (triangles) and GABA + etomidate (squares) for $\alpha4\beta3\delta$ receptors. Error bars denote S.E.M.

$P < 0.001$). The extent of GABA-activated current desensitization at 4 seconds in the presence of etomidate (51% ± 6.0%) was significantly greater than that with GABA alone (20% ± 4.9%) ($P < 0.001$) (Fig. 3B). The weighted time constant (τ_w) of GABA current deactivation in the presence of etomidate (760 ± 83 ms) was significantly greater than that with GABA alone (180 ± 38 ms) ($P < 0.001$) (Fig. 3C).

To test whether the receptor isoform expressed in the inducible cell line is predominantly the $\alpha4\beta3\delta$ receptor, we examined if the etomidate effects on the desensitization and deactivation of $\alpha4\beta3\delta$ receptors differ from those of $\alpha4\beta3$ receptors transiently transfected into HEK293T cells (Fig. 3D). Etomidate at 3.2 μM significantly enhanced the peak current of $\alpha4\beta3$ receptors evoked by 1 mM GABA (360% ± 75%, $n = 10$; $P < 0.01$). However, this peak current enhancement of $\alpha4\beta3$ receptors by etomidate was significantly smaller than that of $\alpha4\beta3\delta$ receptors evoked by etomidate ($P < 0.05$). Interestingly, etomidate significantly decreased GABA current desensitization of $\alpha4\beta3$ receptors from 67% ± 5.5% to 11% ± 3.8% ($P < 0.001$) (Fig. 3E). Etomidate increased the τ_w of GABA current deactivation from 500 ± 123 to 1020 ± 102 ms ($P < 0.01$) for $\alpha4\beta3$ receptors (Fig. 3F).

Etomidate Enhances the Pseudo Steady-State Currents of $\alpha4\beta3\delta$ Receptors. As the $\alpha4\beta3\delta$ receptor is the major isoform to mediate tonic inhibition (Feng and Forman, 2018), we examined the effect of etomidate on the pseudo steady-state currents evoked by prolonged application (30 seconds) of a low concentration of GABA (1 μM) in the absence and presence of etomidate, a surrogate parameter of tonic currents (Fig. 4A). As compared with the pseudo steady-state current evoked by 1 μM GABA, that evoked by 1 μM GABA and 3.2 μM etomidate was significantly augmented (280% ± 51%, $n = 10$; $P < 0.001$) (Fig. 4). To further approach the steady state of receptor activation, application of longer pulses (60 seconds) of a low concentration of GABA in the absence and presence of etomidate was performed. The pseudo steady-state current evoked by 1 μM GABA and 3.2 μM etomidate was also significantly larger than that evoked by 1 μM GABA (410% ± 76%, $n = 9$; $P < 0.001$).

Discussion

In this study, we found that $\alpha4\beta3\delta$ receptors inducibly expressed in HEK293 TetR cells are sensitive to direct activation by either GABA or etomidate. Etomidate at 3.2 μM increases GABA-dependent current amplitude without altering EC_{50}. Etomidate also increases desensitization and slows deactivation of $\alpha4\beta3\delta$ receptors. Using prolonged activation of $\alpha4\beta3\delta$ receptors by a low concentration of GABA to mimic tonic current, we observed that etomidate enhances the pseudo steady-state current of this receptor isoform.

Etomidate Exerts Direct and Modulatory Actions on $\alpha4\beta3\delta$ Receptors. In the current study, we observed that etomidate can directly activate $\alpha4\beta3\delta$ receptors, but the onset of etomidate-evoked currents is slow and the deactivation is fast. The slow activation of receptors by etomidate probably reflects the rate of equilibration in the cytoplasm and membrane by this very hydrophobic drug. We previously demonstrated a similar slow equilibration for a hydrophobic open-channel blocker of nicotinic acetylcholine receptors (Forman, 1999), and others have demonstrated slow access to $GABA_A$ receptors with neurosteroids (Li et al., 2007). It is less clear why the deactivation of etomidate-evoked currents is faster than the activation. Deactivation of currents is probably limited by channel closure, and fast deactivation reflects the low efficacy of etomidate as an $\alpha\beta\delta$ receptor agonist.

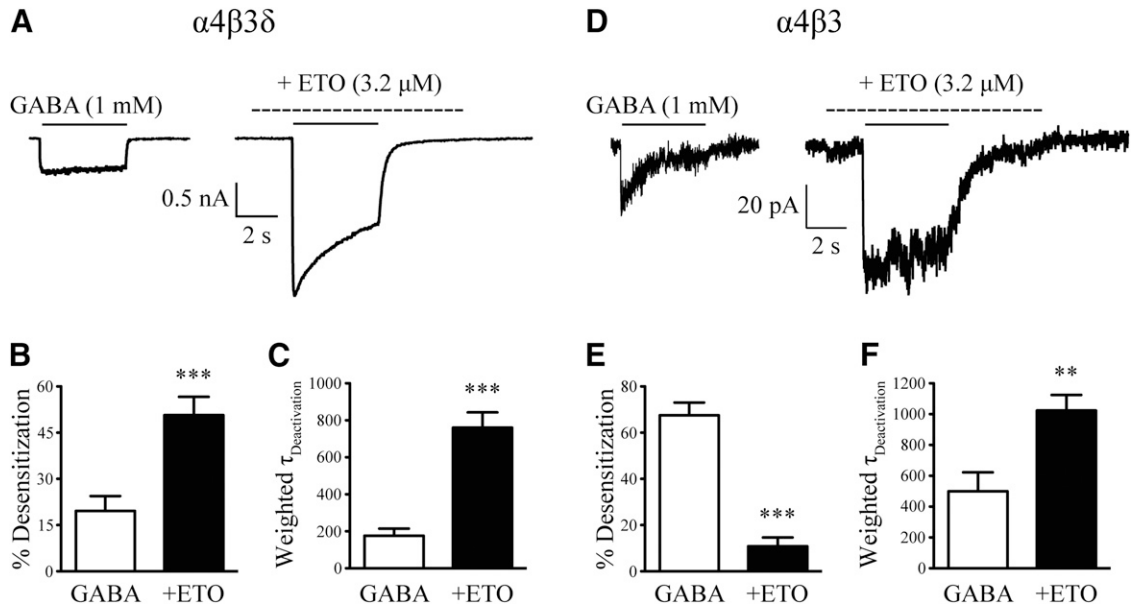

Fig. 3. Etomidate modulates the desensitization and deactivation of $\alpha4\beta3\delta$ and $\alpha4\beta3$ receptors. (A) Representative current traces evoked by saturating GABA (1 mM) or saturating GABA plus etomidate (ETO; 3.2 μM) in $\alpha4\beta3\delta$ receptors inducibly expressed in HEK293 TetR cells. (B) Etomidate increases the extent of desensitization for $\alpha4\beta3\delta$ receptors as compared with that of GABA control current. (C) Etomidate increases the weighted time constant (τ_w) of deactivation for $\alpha4\beta3\delta$ receptors as compared with that of GABA control current. (D) Representative current traces evoked by saturating GABA (1 mM) or saturating GABA plus etomidate (3.2 μM) in $\alpha4\beta3$ receptors transiently expressed in HEK293T cells. (E) Etomidate decreases the extent of desensitization for $\alpha4\beta3$ receptors as compared with that of GABA control current. (F) Etomidate increases the deactivation τ_w of $\alpha4\beta3$ receptors as compared with that of the GABA control current. The solid lines indicate the duration of GABA application (4 seconds), and the dashed line denotes that of etomidate application in (A) and (D). Error bars denote S.E.M. in (B), (C), (E), and (F). $^{**}P < 0.01$; $^{***}P < 0.001$ as compared with GABA control current.

Etomidate is an allosteric coagonist of $\alpha4\beta3\delta$ receptors. The slowing of GABA-elicited current deactivation in the presence of etomidate indicates that etomidate stabilizes open-channel states by slowing their closure (rather than accelerating their opening). The pattern of etomidate effects in $\alpha4\beta3\delta$ GABA concentration responses, where apparent GABA efficacy increases but no large change in apparent GABA potency is observed, is similar to that in $\alpha1\beta3\delta$ (Feng et al., 2014). Equilibrium Monod-Wyman-Changeux models readily explain this pattern when intrinsic GABA efficacy is low. In $\alpha1\beta3\delta$, we estimated that GABA at high concentrations activates less than 5% of receptors, and the lack of EC_{50} shift for $\alpha4\beta3\delta$ suggests that GABA is also a low-efficacy agonist at these receptors.

Of note, the slow equilibration of etomidate with its transmembrane coagonist sites on $\alpha4\beta3\delta$ receptors suggests that our experiments, using simultaneous coapplication of etomidate and GABA, may have underestimated the effects of etomidate in comparison with those using etomidate preapplication before adding GABA. However, desensitization is slow enough that etomidate equilibration is complete under both experimental conditions, and similar effects on desensitization are observed with both approaches. Qualitatively, acceleration of desensitization and slowing of deactivation are evident in both Figs. 2 and 3. Quantitative analysis of etomidate effects on desensitization and deactivation of $\alpha4\beta3\delta$ receptors was based on experiments using etomidate preapplication.

Possible Stoichiometry and Subunit Arrangement of $\alpha4\beta3\delta$ Receptors. Although multiple stoichiometries of free recombinant $\alpha1\beta3\delta$ receptors have been proposed (Kaur et al., 2009), studies suggest that the predominant receptor isoform in free $\alpha1\beta3\delta$ receptors is $\beta3$-$\alpha1$-δ-$\beta3$-$\alpha1$, which shares stoichiometry and subunit arrangement with $\alpha1\beta3\gamma2$ receptors (Feng et al., 2014; Botzolakis et al., 2016). However, anesthetic photolabeling of $\alpha4\beta3\delta$ receptors inducibly expressed in HEK293 TetR cells suggests that $\beta3$-$\beta3$ interfaces are present. If so, possible subunit arrangements of this receptor isoform include $\beta3$-$\beta3$-δ-$\beta3$-$\alpha4$ and $\beta3$-$\beta3$-$\alpha4$-δ-$\alpha4$ (Chiara et al., 2016). Thus, $\alpha4\beta3\delta$ receptors may only have one traditional extracellular β^+/α^- GABA binding site. GABA agonist sites may also be formed at β^+/β^- interfaces, because GABA activates $\beta3$ homomeric $GABA_A$ receptors (Cestari et al.,

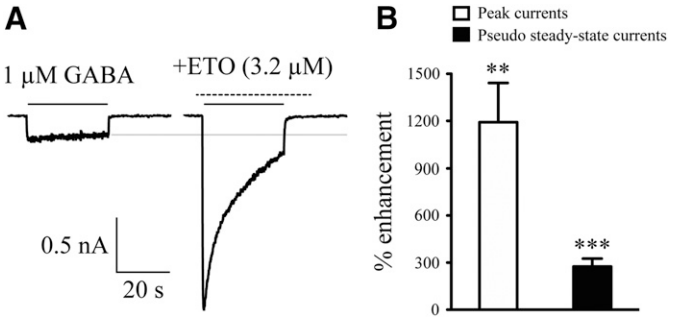

Fig. 4. Etomidate (ETO) augments the pseudo steady-state currents of $\alpha4\beta3\delta$ receptors. (A) Representative current traces evoked by prolonged application (30 seconds) of low concentration of GABA (1 μM) or GABA plus etomidate (3.2 μM) in $\alpha4\beta3\delta$ receptors inducibly expressed in HEK293 TetR cells. The solid lines indicate the duration of GABA application, and the dashed line denotes that of etomidate application. The gray line indicates the pseudo steady-state current amplitude of current elicited by GABA alone. (B) The mean percentage of peak current and pseudo steady-state current enhancement by etomidate in the presence of 1 μM GABA in $\alpha4\beta3\delta$ receptors. Error bars denote S.E.M. $^{**}P < 0.01$ as compared with peak GABA control current; $^{***}P < 0.001$ as compared with pseudo steady-state GABA control current.

1996; Wooltorton et al., 1997). Alternatively, it was also reported that a GABA binding site may exist on the δ subunit interface (Baur et al., 2009; Karim et al., 2012). Similarly, etomidate has a traditional β^+/α^- transmembrane binding site and possibly another β^+/β^- binding site in $\alpha4\beta3\delta$ receptors.

Etomidate Uniquely Modulates the Desensitization of $\alpha4\beta3\delta$ Receptors. Etomidate increased the extent of desensitization of $\alpha4\beta3\delta$ receptors. This observation is in contrast to the effect of etomidate on the desensitization of $\alpha1\beta3\delta$ receptors in a previous study, where etomidate reduced desensitization (Liu et al., 2015). It is unknown why etomidate exerts differential effects on the desensitization of $\alpha4\beta3\delta$ and $\alpha1\beta3\delta$ receptors. However, different general anesthetics exert differential effects on the desensitization of $\alpha1\beta3\delta$ receptors (Feng, 2010). For example, unlike etomidate, barbiturates (Feng et al., 2004; Feng and Macdonald, 2010) and the neurosteroid tetrahydrodeoxycorticosterone (Wohlfarth et al., 2002) increase the desensitization of $\alpha1\beta3\delta$ receptors. These general anesthetics act at different binding sites on $\alpha1\beta2/3\gamma2$ receptors (Jayakar et al., 2015; Feng and Forman, 2018) and probably also on $\alpha1\beta3\delta$ receptors. Thus, distinct anesthetic binding sites may be differentially coupled with receptor desensitization. If $\alpha4\beta3\delta$ and $\alpha1\beta3\delta$ receptors form different etomidate intersubunit binding sites (β^+/β^- vs. β^+/α^-), this could underlie differential effects on desensitization. In addition, the β^+/α^- sites formed by $\alpha1$ and $\alpha4$ subunits may differentially mediate etomidate actions. Azi-etomidate photolabeling identifies both α1M236 (numbering based on mature sequence) and α4M269 (numbering includes leader sequence) as drug contacts (Chiara et al., 2016; Forman and Miller, 2016). Substituted cysteine modification-protection experiments indicated that α1L232 is another etomidate contact residue (Nourmahnad et al., 2016), and its homolog in $\alpha4$ is I265. It is possible that this sequence difference contributes to the opposing effects on desensitization of etomidate in $\alpha1\beta\delta$ and $\alpha4\beta\delta$ receptor isoforms. Additional studies are needed to investigate these possibilities (Feng and Forman, 2018).

Etomidate Augments Pseudo Steady-State Current of $\alpha4\beta3\delta$ Receptors. Etomidate has been shown to enhance the tonic GABA-mediated currents in the brain (Belelli et al., 2005; Kretschmannova et al., 2013; Herd et al., 2014). The $\alpha\beta\delta$ receptor is considered to be the major isoform mediating tonic inhibition (Farrant and Nusser, 2005; Feng and Forman, 2018). Both $\alpha1\beta\delta$ and $\alpha4\beta\delta$ receptors are expressed in the brain (Sur et al., 1999; Jia et al., 2005; Chandra et al., 2006; Drasbek et al., 2007; Glykys et al., 2007). Our previous study demonstrated that etomidate reduces the desensitization of $\alpha1\beta3\delta$ receptors, and thus, enhancement of the pseudo steady-state currents is greater than that of peak currents in this receptor isoform (Liu et al., 2015). However, in $\alpha4\beta3\delta$ receptors, etomidate enhancement of pseudo steady-state currents is less than that of peak currents after rapid application of 1 μM GABA because of increased desensitization. Nonetheless, the persistent increase in pseudo steady-state currents suggests that $\alpha4\beta3\delta$ receptors can contribute to the central nervous system effects of etomidate.

Authorship Contributions

Participated in research design: Forman, Feng.

Conducted experiments: Liao, Liu, Jounaidi.

Performed data analysis: Liao, Liu, Feng.

Wrote or contributed to the writing of the manuscript: Forman, Feng.

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Address correspondence to: Dr. Hua-Jun Feng, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Edwards 505, Boston, MA 02114. E-mail: feng.huajun@mgh.harvard.edu; or Dr. Stuart A. Forman, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Edwards 505, Boston, MA 02114. E-mail: saforman@mgh.harvard.edu