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Etomidate, propofol and the neurosteroid THDOC increase the GABA efficacy of recombinant α4β3δ and α4β3 $GABA_A$ receptors expressed in HEK cells

Pratap Meera [a], Richard W. Olsen [b], Thomas S. Otis [a], Martin Wallner [b,*]

[a] Department of Neurobiology, Geffen School of Medicine, University of California, Los Angeles, USA
[b] Department of Molecular and Medical Pharmacology, Geffen School of Medicine, University of California, Los Angeles, CA 90095-1735, USA

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ABSTRACT

General anesthetics, once thought to exert their effects through non-specific membrane effects, have highly specific ion channel targets that can silence neuronal populations in the nervous system, thereby causing unconsciousness and immobility, characteristic of general anesthesia. Inhibitory $GABA_A$ receptors ($GABA_A$Rs), particularly highly GABA-sensitive extrasynaptic receptor subtypes that give rise to sustained inhibitory currents, are uniquely sensitive to $GABA_A$R-active anesthetics. A prominent population of extrasynaptic $GABA_A$Rs is made up of α4, β2 or β3, and δ subunits. Considering the demonstrated importance of GABA receptor β3 subunits for *in vivo* anesthetic effects of etomidate and propofol, we decided to investigate the effects of GABA anesthetics on "extrasynaptic" α4β3δ and also binary α4β3 receptors expressed in human embryonic kidney (HEK) cells. Consistent with previous work on similar receptor subtypes we show that maximal GABA currents through "extrasynaptic" α4β3δ receptors, receptors defined by sensitivity to EtOH (30 mM) and the β-carboline β-CCE (1 μM), are enhanced by the $GABA_A$R-active anesthetics etomidate, propofol, and the neurosteroid anesthetic THDOC. Furthermore, we show that receptors formed by α4β3 subunits alone also show high GABA sensitivity and that saturating GABA responses of α4β3 receptors are increased to the same extent by etomidate, propofol, and THDOC as are α4β3δ receptors. Therefore, both α4β3 and α4β3δ receptors show low GABA efficacy, and GABA is also a partial agonist on certain binary αβ receptor subtypes. Increasing GABA efficacy on α4/6β3δ and α4β3 receptors is likely to make an important contribution to the anesthetic effects of etomidate, propofol and the neurosteroid THDOC.

1. Introduction

Synaptic $GABA_A$ receptors contain γ subunits (with the γ2 subunit being the most abundant subtype) and are thought to be located to synapses through interactions of the γ subunit with accessory proteins like GABARAP (Wang et al., 1999) and gephryin (Kneussel and Betz, 2000). This implies that most $GABA_A$ receptors without γ subunits are found outside of synapses. The presence of a γ subunit also makes $GABA_A$Rs less sensitive to GABA, when compared to their matching αβ counterparts (Baburin et al., 2008). Therefore, it is thought that activation of most γ subunit-containing "synaptic" $GABA_A$Rs require relatively high GABA concentrations supplied by classical synaptic vesicular GABAergic neurotransmission. Given the relatively low GABA sensitivity of γ subunit-containing receptors it seems likely that γ subunit-containing receptors are usually inactive at the low ambient "extrasynaptic" concentrations of GABA (≤1 μM) that are determined largely by the activity of GABA transporters (Wu et al., 2007). Tonic currents mediated by α5 subunit-containing $GABA_A$Rs in CA1 hippocampal pyramidal neurons might be an exception (Glykys et al., 2008), as these receptors have been proposed to be composed of α5β3γ2 subunits (Caraiscos et al., 2004).

Certain $GABA_A$R subtypes such as those containing δ subunits are excluded from synapses and show an extrasynaptic or perisynaptic localization (Farrant and Nusser, 2005). In contrast to the effects of γ2 subunits, incorporation of δ subunits into functional receptors is believed to increase GABA potency ($EC_{50} < 1$ μM) when compared to matching αβ $GABA_A$Rs. This high GABA potency allows δ subunit-containing receptors to open at low ambient GABA concentrations, a feature that (together with slow desensitization) makes δ subunit-containing receptors uniquely suited to mediate a constant (tonic) inhibitory activity that can potently suppress neuronal excitation. The importance of ambient GABA levels and tonic GABA currents in the control of normal and pathological

Abbreviations: GABA, γ-aminobutyric acid; $GABA_A$R, type A GABA receptor; HEK, human embryonic kidney; β-CCE, β-carboline-3-carboxyethyl ester; EtOH, ethanol; THDOC, tetrahydrodeoxycorticosterone or 3α,21-dihydroxy-5α-preganan-20-one.

* Corresponding author. Tel.: +1 310 825 5748.
E-mail address: mwallner@mednet.ucla.edu (M. Wallner).

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neuronal activity (Richerson, 2004) as well as the finding that extrasynaptic $GABA_A$Rs are important mediators of alcohol (Hanchar et al., 2005; Wallner et al., 2006a) and gaboxadol (Chandra et al., 2006) actions, makes it likely that extrasynaptic receptors play an important role in controlling neuronal excitability even in the absence of exogenous modulators. In addition, many medications that are used to control hyperexcitability phenomena like convulsions, epilepsy, and tremor directly or indirectly (e.g., by increasing extrasynaptic GABA) augment tonic inhibition (Richerson, 2004).

It has been shown in neurons in slices that tonic GABA currents mediated by δ subunit-containing receptors are distinguished from their synaptic counterparts by several pharmacological features: (1) they are enhanced by low nanomolar concentrations of the neurosteroid THDOC (Stell et al., 2003); (2) they are enhanced by ethanol at low inebriating (3–30 mM) concentrations (Hanchar et al., 2005; Wei et al., 2004); and (3) they are activated by high nanomolar concentrations of the GABA-analog THIP/gaboxadol (Chandra et al., 2006; Cope et al., 2005). Given that THDOC, ethanol, and THIP have anesthetic-like actions (lead to "loss of righting reflex" in rodents at appropriate doses), the high potency and efficacy of these drugs on δ subunit-containing receptors suggest that δ subunit-containing receptors can mediate functional effects of $GABA_A$R-active anesthetics. In addition, while δ subunit-containing receptors are uniquely sensitive to GABA, it has been shown in a number of studies on recombinant δ subunit-containing receptors that etomidate, propofol, THDOC and barbiturates lead to increases in peak currents even at saturating GABA concentrations (Bianchi and Macdonald, 2003; Feng et al., 2004; Wallner et al., 2003; Wohlfarth et al., 2002; Zheleznova et al., 2008). This indicates that δ subunit-containing receptor subtypes, while showing high potency for GABA, have a low GABA efficacy, likely because of inefficient coupling of GABA binding to pore opening. In other words, GABA can be considered a partial agonist on these low efficacy $GABA_A$R subtypes (Bianchi and Macdonald, 2003; Wallner et al., 2003). The low GABA efficacy on δ subunit-containing receptors seen in recombinant systems (particularly with α1 subunits and in the oocyte expression system) explains some of the difficulty in expressing these receptors in recombinant systems, with one recent report stating that α1β2δ receptors expressed in oocytes are essentially silent receptors which can be "recruited" by tracazolate and the neurosteroid THDOC (Zheleznova et al., 2008).

Besides δ subunit-containing receptors, there is evidence for additional $GABA_A$R subtypes formed by receptors composed of binary αβ combinations without δ, γ, or ϵ subunits in the pentamer. Particularly interesting in this respect are biochemical data showing that only 7% of α4 subunits are associated with δ subunits, whereas around 50% of α4 subunit-containing receptors do not contain γ or δ subunits (Bencsits et al., 1999). It seems likely that the majority of these α4 subunit-containing receptors without γ or δ subunits are composed of α4 and β subunits alone (Bencsits et al., 1999). Such binary, αβ receptor subtypes also have been proposed to be present in cultured hippocampal neurons where they mediate certain forms of tonic inhibition (Mortensen and Smart, 2006) and it seems likely that much of the GABA response recorded from neurons derived from γ2 subunit knock-out mice is mediated by αβ receptor subtypes (Günther et al., 1995).

GABA receptors containing β3 subunits are important mediators of etomidate and propofol anesthetic effects *in vivo*, because it has been shown that mice with the knock-in point mutation β3N265M lack the immobilizing effects of etomidate and propofol in response to painful stimuli (Jurd et al., 2003). In contrast, a similar knock-in point mutation at the homologous position in the β2 subunit that drastically reduces etomidate effects in β2 subunit-containing receptors (β2N265S, a change in β2 to the residue present in the "etomidate-insensitive" β1 subunit (Belelli et al., 1997)), reduces the sedative but not the immobilizing and hypnotic etomidate effects (Reynolds et al., 2003). One factor that may account for the importance of β3 subunit-containing receptors in meditating anesthetic actions is the association of β3 subunits with extrasynaptic receptors, a finding supported by the correlation of alcohol sensitivity of β3 subunit-containing receptors in recombinant systems (Wallner et al., 2003) with alcohol sensitivity of native tonic δ receptor-mediated GABA currents (Hanchar et al., 2005; Wei et al., 2004). Another factor may be that β3 subunit-containing $GABA_A$R subtypes could be key components in specific neuronal circuits that are particularly important in mediating anesthetic actions (Grasshoff et al., 2006).

Here we compare receptors formed by α4β3 subunits alone with those formed by α4β3δ subunits. We find that α4β3 receptors show only slightly lower GABA sensitivity when compared to α4β3δ receptors (α4β3 GABA $EC_{50} = 1.1$ μM, α4β3δ GABA $EC_{50} = 0.5$ μM), a finding consistent with the notion that binary α4β3 receptors present in native neurons could mediate unique forms of tonic inhibition. Further we show that peak GABA responses, not only on α4β3δ, but also on binary α4β3 receptors are enhanced by $GABA_A$R-active anesthetics etomidate, propofol, and THDOC. Thus, the suggestion that these anesthetics act by increasing GABA efficacy can be extended to include binary α4β3 receptors and possibly other "binary" αβ $GABA_A$R subtypes. Our results are consistent with the hypothesis that binary αβ receptors could contribute not only to tonic GABA currents, but also that such receptors could potentially make important contributions to the actions of $GABA_A$R-active anesthetics.

2. Results

2.1. α4β3 and α4β3δ Receptors expressed in HEK cells are sensitive to low "extrasynaptic" [GABA]

$GABA_A$Rs composed only with α and β subunits may exist on native neurons and are readily expressed in recombinant systems; indeed evidence suggests that they are often a contaminating species when γ2, δ or ϵ subunit-containing receptors are the desired subject of study in recombinant expression (Boileau et al., 2002; Baburin et al., 2008; Olsen et al., 2007). Most native δ subunit-containing $GABA_A$Rs are composed with α4 or α6, and either β2 or β3 subunits. Considering that β3 has been implicated as important for anesthetic actions and low dose ethanol sensitivity, we decided to compare the GABA sensitivity and modulation by GABA anesthetics of α4β3 and α4β3δ $GABA_A$Rs.

Fig. 1 shows that receptors formed by α4β3δ subunits in HEK cells are half-maximally activated at a GABA concentration of ~500 nM, an EC_{50} only slightly higher than our previous (lower) estimate (~200 nM) of extrasynaptic [GABA] in cerebellar granule cells in perfused slices (Santhakumar et al., 2006). Binary α4β3 receptors also respond to low GABA concentrations, although slightly less potently ($EC_{50} = 1.1$ μM, 300 nM is ~GABA EC_{20} at binary α4β3 receptors). This small, yet highly significant shift in GABA sensitivity indicates that δ subunit expression indeed leads to increased GABA sensitivity and this is opposite to the reduced GABA sensitivity generally observed with γ subunit incorporation.

We showed previously with recombinant expression in oocytes that expression of the δ subunit makes α4β3δ and α6β3δ receptors sensitive to inebriating (3–30 mM) concentrations of ethanol, whereas α4β3 receptors are insensitive to EtOH at these same low concentrations (Hanchar et al., 2005; Wallner et al., 2003). We therefore investigated in α4β3δ-transfected HEK cells if GABA responses are ethanol sensitive. Fig. 2 shows representative recordings from α4β3δ- and α4β3-transfected cells in which various pharmacological features (sensitivity to 30 mM ethanol, 1 μM β-CCE, and 1 μM Zn^{2+}, co-applied with GABA) were tested. As