Positive modulation of $GABA_A$ receptor currents and anesthesia by cyclohexanol analogs

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Abstract

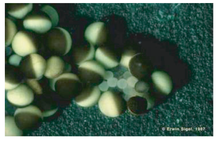
$GABA_A$ receptors meet all the pharmacological criteria required to be considered important general anesthetic targets. In the following study, effects of various cyclohexanol analogs were investigated on recombinant human γ-aminobutyric acid ($GABA_A$, α1β2γ2s) receptors expressed in Xenopus oocytes. Submaximal EC_{20} GABA currents were typically enhanced by co-applications of 1-300 μM cyclohexanols analogs. For instance, at 30 μM 2,6-diisopropylcyclohexanol (a novel compound), GABA responses were enhanced ~4-fold with the potentiating effects for 2,6-diisopropylcyclohexanol = 2,6-dimethylcyclohexanol > 2,6-di-s-butylcyclohexanol >2,6 di-ethylcylohexanol > 2-methylcyclohexanol > 2,6-di-t-butylcyclohexanol > cyclohexanol > cylopentanol > 4-t-butylcylohexanol. We then tested the potencies of the cyclohexanol analogs as general anesthetics using a tadpole assay. Both 2,6-diisopropylcyclohexanol and 2,6-dimethylcyclohexanol were effective as anesthetics with EC_{50}s of 14.0 μM and 13.1 μM respectively, while other cyclohexanol analogs with bulkier side chains were less potent. Cyclohexanol, cyclopentanol, and 2 methylcyclohexanol were not anesthetic. In conclusion, our data indicate that some cyclohexanol analogs are both positive modulators of $GABA_A$ receptors currents and anesthetics. The positioning and size of the alkyl groups at the 2 and 6 positions on the cyclohexanol ring are critical determinants of this activity.

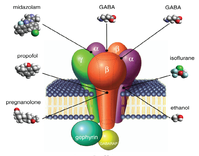
Introduction

- $GABA_A$ receptors are the principal ionotropic receptors for inhibitory neurotransmission in the mammalian brain and are targets for modulation by sedatives, anxiolytics, general anesthetics, and convulsant agents.
- We investigated modulation of $GABA_A$ receptors currents and anesthetic potency for cyclohexanols analogs.

Materials and Methods

- Xenopus laevis oocytes harvested through laparotomy.
- cDNAs encoding for α1β2γ2s subunits of human $GABA_A$ receptor (ratio of 1:1:0.5, 30ng/ml) were injected into the nucleus of oocytes.
- Recordings used standard two-electrode voltage clamp, routinely clamping at -50mV.
- Oocytes were superfused with recording solution including 30μM GABA (~EC_{20}) and dilutions of drugs (3-300 μM).
- Tadpole assays were performed to assess the anesthetic potency. Tadpoles (n=30 per compound) were exposed to 0.3-300 μM of a drug
- Experimenter (blinded to the conditions) assessed numbers of anesthetized tadpoles (loss of swimming reflex) over a 2-hour period.
- Data were analyzed using the Ward equation for quantal analyses





In Vitro
$GABA_A$ Receptor Assay

Results

Figure 1. Enhancement of a sub-maximal (EC_{20}) GABA current by cyclohexanols

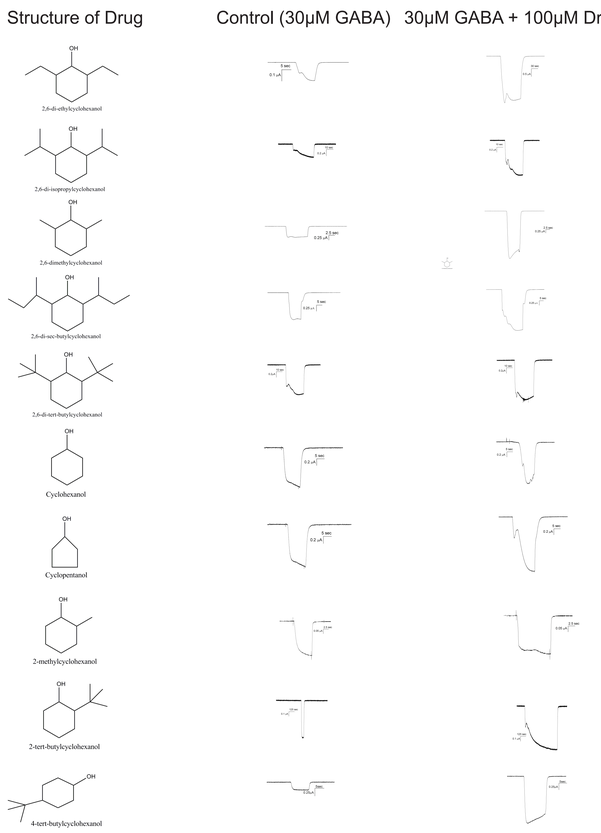
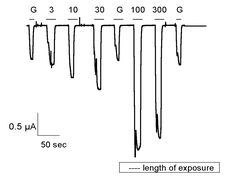
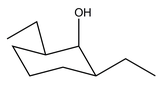


Figure 2. Enhancement of sub-maximal (EC_{20}) GABA current by increasing concentrations of 2,6 diethylcyclohexanol





Results

Figure 3. Concentration-response for modulation of GABA currents by cyclohexanols analogs

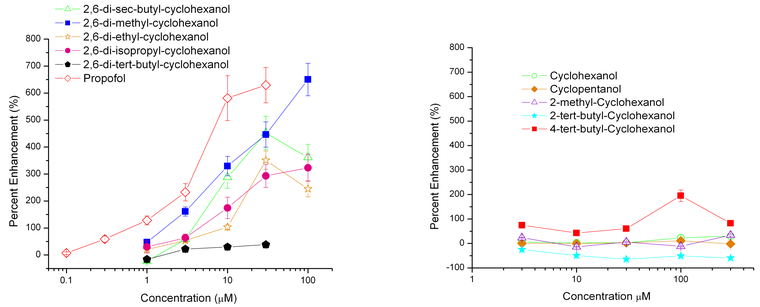
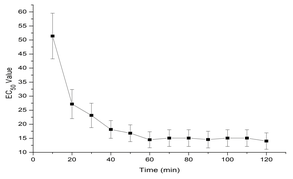


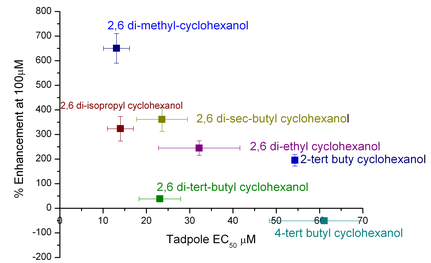
Figure 4. Anesthetic potency (EC50) of 2,6-di-isopropyl cyclohexanol in tadpoles over time



[Figure 4] EC_{50} values for 2,6-di-isopropyl-cyclohexanol over time for tadpole anesthesia *Plot shows the decreasing EC_{50} values calculated by the Ward equation over the time of the experiment. 2,6-DIPCH has an EC_{50} value of 14 ± 3 μM (n=30) after 120 minutes.*

In Vivo
Tadpole Assay

Figure 5. Anesthetic potency (EC50) *In Vivo* vs. Anesthetic Potency (% Enhancement) *In Vitro*



Conclusions

- Cyclohexanols with alkyl chains in the 2,6 position enhanced GABA currents with potency while those without 2,6 alkyl groups did not (Fig. 1, 2, and 3)

- The 2,6 series of cyclohexanols produced measurable levels of anesthetic action in tadpoles while cyclohexanol, cyclopentanol, and 2-methycyclohexanol were not anesthetic (Fig. 5)

- Only compounds that demonstrated marked positive modulation of GABA currents exhibited any anesthetic potency in the tadpole assay (Fig. 3 and 5)

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