Substituted Cysteine Modification and Protection with Alkyl-MTS Reagents Estimates Etomidate-to-Residue Distances in $GABA_A$ Receptors.

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Introduction: Etomidate (ETO) acts at transmembrane β+/α- interfaces of $GABA_A$ receptors. Amino acid residues lining the ETO site, including β3M286, were identified through photolabeling and Substituted Cysteine Modification and Protection (SCAMP) methods. The SCAMP studies used 9 Å long, p-chloromercuribenzene sulfonate (pCMBS). We hypothesized that SCAMP using a series of n-alkyl-methanethiosulfonate (alkyl-MTS) reagents could provide more precise information about the distance between ETO and nearby amino acid residues. We tested this strategy in α1β3M286Cγ2L $GABA_A$ receptors.
Methods: Messenger RNA mixtures encoding α1β3M286Cγ2L $GABA_A$ receptor subunits (1:1:5 ratio) were injected into *Xenopus* oocytes, which were used in two-electrode voltage-clamp electrophysiologic experiments (20 °C; Vm = -50 mV) 18 to 48 hours later. Covalent modification by alkyl-MTS reagents at β3M286C residues was measured as changes in both the low:high GABA current response ratio [10 μM GABA (~ EC2-5) vs. 10 mM GABA] and ETO enhancement ratio [10 μM GABA + 10 μM ETO vs. 10 μM GABA]. Currents were measured in duplicate both before and after oocyte exposure to alkyl-MTS reagents + 3 mM GABA for 30 s, using 3 mM GABA alone as a control exposure. Averaged results after modification were normalized to pre-exposure average results in the same oocyte. Alkyl-MTS reagents that produced statistically significant and concentration-dependent changes in low:high GABA response ratios when compared to were further studied to determine if modification effects were altered by adding 300 μM ETO during alkyl-MTS + 3 mM GABA exposure. We inferred that ETO sterically interacted with the MTS reagent if significant inhibition of modification effects were found. Comparisons were based on Student's t-tests with $n \geq 5$ oocytes per condition.
Results: Alkyl-MTS reagents produced no persistent effects in wild-type α1β3γ2L $GABA_A$ receptors. In α1β3M286Cγ2L receptors, low:high GABA response ratios were unaffected by methyl-MTS or ethyl-MTS at up to 1 mM. Propyl-MTS, butyl-MTS, hexyl-MTS, octyl-MTS, and decyl-MTS all enhanced low:high GABA response ratios ($P = 0.0042$, 0.0006, <0.0001, 0.0008, 0.0034 respectively). ETO enhancement was unaffected by methyl-MTS, increased by ethyl-MTS ($P = 0.0247$), and reduced by larger alkyl-MTS reagents. ETO at 300 μM reduced the effects of exposure to propyl-MTS, butyl-MTS, hexyl-MTS, and octyl-MTS ($P= 0.0032$, 0.0011, <0.0001, 0.0219 respectively).
Conclusion:
We observed a 'cut on' for β3M286C modification effects on both GABA and ETO sensitivity between ethyl-MTS and propyl-MTS, and also found that receptor-bound ETO blocked modification by propyl-MTS and larger reagents. Accounting for the different side-chain lengths of methionine and cysteine, our results indicate that ETO is located 1.7 to 3.0 Å from β3M286 (Fig. 1). SCAMP with alkyl-MTS reagents may be more widely useful as a 'molecular ruler' to assess distances between ETO and other anesthetics and nearby residues in $GABA_A$ receptors.

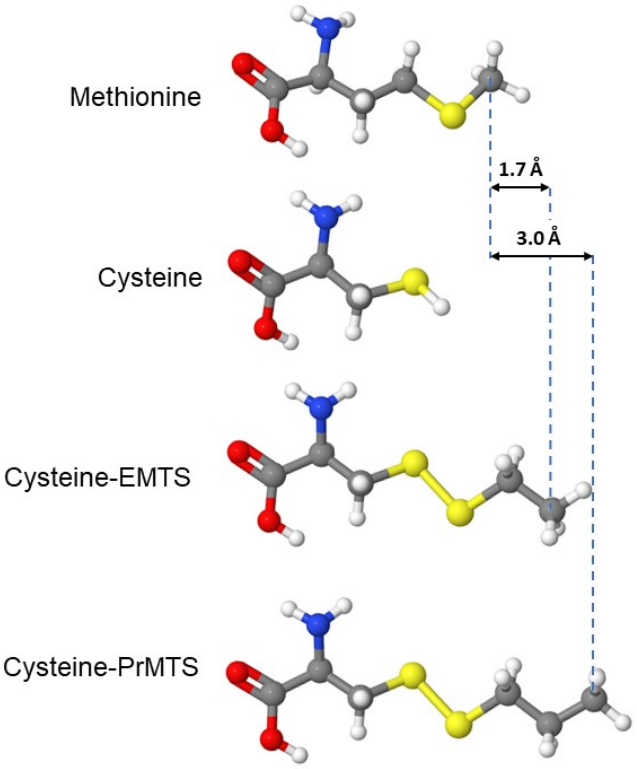

Fig 1. Interpretation of alkyl-MTS effects on α1β3M286Cγ2L $GABA_A$Rs. Modification with reagents longer than ethyl-MTS produce effects on GABA and ETO sensitivity that are inhibited when ETO is present during exposure, suggesting overlap with receptor-bound ETO.