Anesthesia Sensitivity in $GABA_A$ β3 Subunit Mutant Zebrafish

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Introduction: The anesthetic action target γ-aminobutyric acid type A receptor ($GABA_AR$) exhibits diverse subunit heterogeneity. Based on studies in transgenic mice, the β3 subunit of $GABA_AR$ mediates anesthetic effect of etomidate, propofol, and pentobarbital. We have applied the zebrafish model to characterize general anesthetic potencies and drug discovery. In this study, we generated global $GABA_AR$-β3 mutants using CRISPR-Cas9 and compared their sensitivity to various general anesthetics with that of wild-type (WT) zebrafish.

Methods: CRISPR-Cas9 gene targeting and embryonic stem cell technologies were used to create β3-/- zebrafish. Guide RNA targeting on exon 7 of β3 subunit was injected into one-cell stage embryos. Anesthetic potency of etomidate, propofol, alphaxalone, ketamine, pentobarbital, tricaine, ethanol and butanol were determined using photomotor response in 7-day post fertilization larvae. Sedative effects were also measured with pre-flash spontaneous activity. 16~24 larvae/ concentration were included for each assay. Individual larvae movements were tracked using a video system (ViewPoint Zebralab), which also coordinated stimuli. Non-linear least squares fits to logistic equations were used to derive EC_{50} from potency test. Extra sum-of-squares F test was used to determine whether EC_{50} from β3-/- and WT were statistically different.

Results: Genome analysis revealed a 10bp insertion in exon 7 of $GABA_AR$-β3, introducing a premature stop-codon on mRNA and thereby truncation of protein from extracellular domain of the receptor. $GABA_AR$-β3 mutants did not differ in embryogenesis and fertility from WT, but were significantly more resistant to anesthetic effect of etomidate, propofol, pentobarbital, butanol and more sensitive to ethanol. No differences in anesthetic effect were noted in ketamine and tricaine (See Table 1). Similar changes in sedative effects were also observed except for ketamine where β3 -/- were more resistant to ketamine (β3 -/- [EC_{50}] of 0.1μM *vs.* WT [EC_{50}] of 0.04μM, $P<0.0001$).

Conclusions: Our findings demonstrate the anesthetic sensitivity phenotype of GABR-β3 mutant zebrafish, supporting the utility of this model to investigate mechanism of novel anesthetics acting through $GABA_AR$-β3 and normal neuro-physiology process.

Table 1: Comparison of anesthetics potency between β3 -/- and WT

Anesthetics	**β3 -/- [EC_{50}]**	**WT [EC_{50}]**	***P* value**
etomidate	1.12μM	0.55 μM	0.0001
propofol	1.03μM	0.72 μM	0.0029
pentobarbital	229.5μM	103.4 μM	0.0001
butanol	7.8mM	1.9mM	0.0001
ethanol	235.0mM	375.6mM	0.0013
alphaxalone	1.15μM	1.23μM	0.83
ketamine	55.7μM	50.6μM	0.59
tricaine	99.5μM	76.9μM	0.1166